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Original Article

Potential salivary and serum biomarkers for burning mouth syndrome and their relationship with anxiety/depression

Ying Zhang [a,b†], Sai Ye [a,b†], Yangqing Zhang [c], Hong Sun [d], Xiaoxian Zhao [a,b], Xuemin Shen [a,b**], Lan Wu [a,b*]

[a] *Department of Oral Mucosal Diseases, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[b] *Shanghai Key Laboratory of Stomatology, College of Stomatology, National Center for Stomatology, National Clinical Research Center for Oral Diseases, Shanghai Jiao Tong University, Shanghai, China*
[c] *No.2 High School of East China Normal University, Shanghai, China*
[d] *Department of Laboratory Medicine, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

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KEYWORDS
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Abstract *Background/purpose:* The pathophysiology of burning mouth syndrome (BMS), although considered a multifactorial etiology including psychological factors, is still not well understood. Hence, this study aimed to investigate the potential usage of salivary and serum biomarkers, including brain-derived neurotrophic factor (BDNF), interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α), in diagnosing BMS and their correlations with anxiety/depression.
Materials and methods: 45 BMS patients and 14 healthy volunteers were enrolled. The patients were divided into BMS with anxiety/depression group and BMS without anxiety/depression group according to the scores of the Zung Self-rating Anxiety Scale (SAS) and Zung Self-rating Depression Scale (SDS). Additionally, concentrations of BDNF, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α in saliva and those in serum among the patients and healthy volunteers were assessed by multiplex assay using Luminex 200TM system and Enzyme-linked immunosorbent assay (ELISA), respectively.
Results: Among all the serum biomarkers, only BDNF showed a statistically significant decrease in the patients than the healthy volunteers ($P < 0.05$). Regarding saliva biomarkers, BDNF, IL-1β, and IL-8 all exhibited a statistically significant increase in all the BMS patients versus the healthy volunteers ($P < 0.05$) but only BDNF was significantly different between patients with

* Corresponding author. Department of Oral Mucosal Diseases, Shanghai Ninth People's Hospital, 500 Quxi Road, Shanghai, 200011, China.
** Corresponding author. Department of Oral Mucosal Diseases, Shanghai Ninth People's Hospital, 500 Quxi Road, Shanghai, 200011, China.
E-mail addresses: kiyoshen@163.com (X. Shen), teana_wu@sina.com (L. Wu).
† Y. Zhang and S. Ye contributed equally to this work.

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anxiety/depression and healthy individuals when considering anxiety/depression. Among BMS patients with anxiety/depression, saliva TNF-α had positive associations with other biomarkers including BDNF, IFN-γ, IL-1β, IL-6, and IL-8 ($P < 0.05$).
Conclusion: The increased concentration of saliva BDNF holds strong potential for diagnosing BMS and the elevated level of saliva TNF-α is crucial in identifying BMS patients with anxiety/depression.

Introduction

Burning mouth syndrome (BMS), primarily a diagnosis of exclusion based on subjective symptoms, is a chronic condition characterized by a burning sensation of the intraoral mucosa in the absence of a local or systemic cause.[1] The most common symptom of BMS is a burning sensation, but other symptoms including dryness, uncomfortable sensations in the mouth and changes to taste, allodynia, pricking pain, tingling, electrical discharges, numbness, and itching can also occur.[2] The characteristic burning sensation of BMS is typically bilateral and is most commonly experienced on the tongue, although it can be experienced anywhere in the intraoral mucosa, including the lips, hard palate, gingiva, and buccal mucosa.[3,4] The worldwide prevalence of BMS was 1.73% in population-based studies and most cases of BMS occur in females after the age of 50.[1,5] The onset of BMS is most often spontaneous and symptoms progress gradually in most patients, often persisting for several years. Less than 3% of affected individuals got complete spontaneous resolution after 5 years, and less than 30% of patients with BMS show moderate symptom improvement with or without treatment.[3,6] Since persistent and suffered long term, BMS can lead to a reduced quality of life (QoL) in the affected individuals, who can become high consumers of health care resources as a result.[1,2]

The etiopathogenesis of BMS is still not well understood, although widely considered as a multifactorial etiology involving peripheral small fiber neuropathy, dysfunction in the brain network, and psychological factors.[3,5,7–10] Diagnosing BMS may be intractable even for the physician given our limited knowledge of its pathogenesis. Importantly, biomarkers are the molecular signatures and indicators of normal biological, pathological processes, and pharmacological responses to treatment hence may provide useful information for the detection, diagnosis, and prognosis of the disease. The diagnostic modality in the field of molecular biology has led to the discovery and potential of salivary and blood biomarkers for the detection of early oral cancer, Alzheimer's disease, psychological disorders, osteoarthritis, etc.[11–14] Notably, the use of saliva in the search for new clinical markers is a promising approach because of its noninvasive sampling and easy collection methods.

Therefore, in this study, we investigated the basic symptoms and the anxiety/depression status among BMS patients. Meanwhile, the correlations between anxiety/depression, salivary biomarkers, and serum biomarkers were also studied. The current study aims to explore the potential usage of salivary and serum biomarkers in the diagnosis of BMS.

Materials and methods

Procedures and participants

Participants of the study were recruited among individuals who visited the Department of Oral Mucosal Diseases at Shanghai Ninth People's Hospital between September 2021 and September 2022. The participants were grouped as either the case (patients with BMS) or the control (healthy) group.

Inclusion criteria for the case group were: (1) age over 18; (2) had no difficulty communicating in Chinese; and (3) had a diagnosis of BMS. The diagnosis was based on the definition of "idiopathic orofacial pain with intraoral burning or dysesthesia recurring daily for more than 2 h per day and more than 3 months, without any identifiable causative lesions, with or without somatosensory changes" proposed by the International Classification of Orofacial Pain, 2020. The unexplained nature of burning symptoms was confirmed by a comprehensive step-by-step examination and necessary laboratory tests. Exclusion criteria for the case group were: (1) deficiencies in iron, folic acid, or vitamin B12; (2) the presence of systemic diseases such as Diabetes, Sjögren's syndrome, liver or kidney diseases, etc.; (3) the presence of other oral diseases such as oral candidiasis, oral lichen planus, denture-induced damage, etc.; (4) usage of antibiotics within the past one month or immunosuppressive drugs within the past three months or cytotoxic drugs over 3 months.

Informed written consent was obtained from all the participants, and the study protocol was approved by the Ethical Committee of Shanghai Ninth People's Hospital (SH9H-2021-T199-2).

Parameters and measures

First of all, baseline characteristics including gender, age, symptoms, location, and duration were collected. Furthermore, the Visual Analogue Scale (VAS), ranging from 0 to 10 (anchored by 0 = no pain and 10 = very severe pain), was used to estimate the intensity of pain among BMS patients. The pain level ratings were classified as mild (1–3), moderate (4–6), or severe (7–10).[15,16]

Additionally, Zung Self-rating Anxiety Scale (SAS) and Zung Self-rating Depression Scale (SDS) were respectively used to assess anxiety and depression levels in BMS patients. Both are 20 items self-report assessment tools designed to measure anxiety/depression levels. Each item score was ranked from one (none or a little of the time) to four (most or all of the time). Among 20 items, half the items were positively worded (e.g. "I eat as much as I used to") and they were scored in the opposite direction. The final index score was converted by multiplying the raw score by 1.25 and then rounding off decimal places. The SAS scores were divided into four categories: normal range ($\leq$49), mild anxiety (50—59), moderate anxiety (60—74), and severe anxiety ($\geq$75). The severity of depression was categorized according to the index score: no depression ($<$50), mild depression (50—59), moderate depression (60—69), and severe depression ($\geq$70). SAS internal consistency reliability was 0.66—0.80 and the Cronbach's α was 0.87.[17,18]

Before saliva sample collection, the participants were instructed to rinse their mouths thoroughly for 2 min. Subsequently, the samples were immediately placed in coolers and transported to the laboratory, where saliva was vortexed and centrifuged (1000 rpm, 5 min). Next, the supernatant was transferred into polypropylene tubes and stored at -80 °C. The supernate was used to test concentrations of brain-derived neurotrophic factor (BDNF), interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) by multiplex assay using the Human Custom Procartaplex Assay Kit (Invitrogen, Carlsbad, CA, USA) and Luminex 200TM system (Luminex Corporation, Austin, TX, USA) according to the manufacturer' s instructions. Besides, the serum was obtained from the peripheral blood samples by centrifuge at 3500 rpm for 10 min and stored at -80 °C. Once finishing the sample collection, Enzyme-linked immunosorbent assay (ELISA) kits (MultiSciences Biotech Co., Ltd, Hangzhou, China) were used to measure the levels of inflammatory cytokines, including BDNF, IFN-γ, IL-1β, IL-6, IL-8, TNF-α.

Statistical analysis

All data were analyzed using GraphPad Prism version 9.0 (GraphPad, San Diego, CA, USA) and SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Qualitative data were expressed as the number of cases as well as proportions and analyzed using the χ^2 test. Quantitative data were expressed as mean $\pm$ SD. Differences between groups were analyzed using an independent samples *t*-test. Correlations between VAS, SAS, SDS, and the six biomarkers in saliva or serum of the same group were analyzed by Spearman's correlation analysis. The significance level was established at $P < 0.05$.

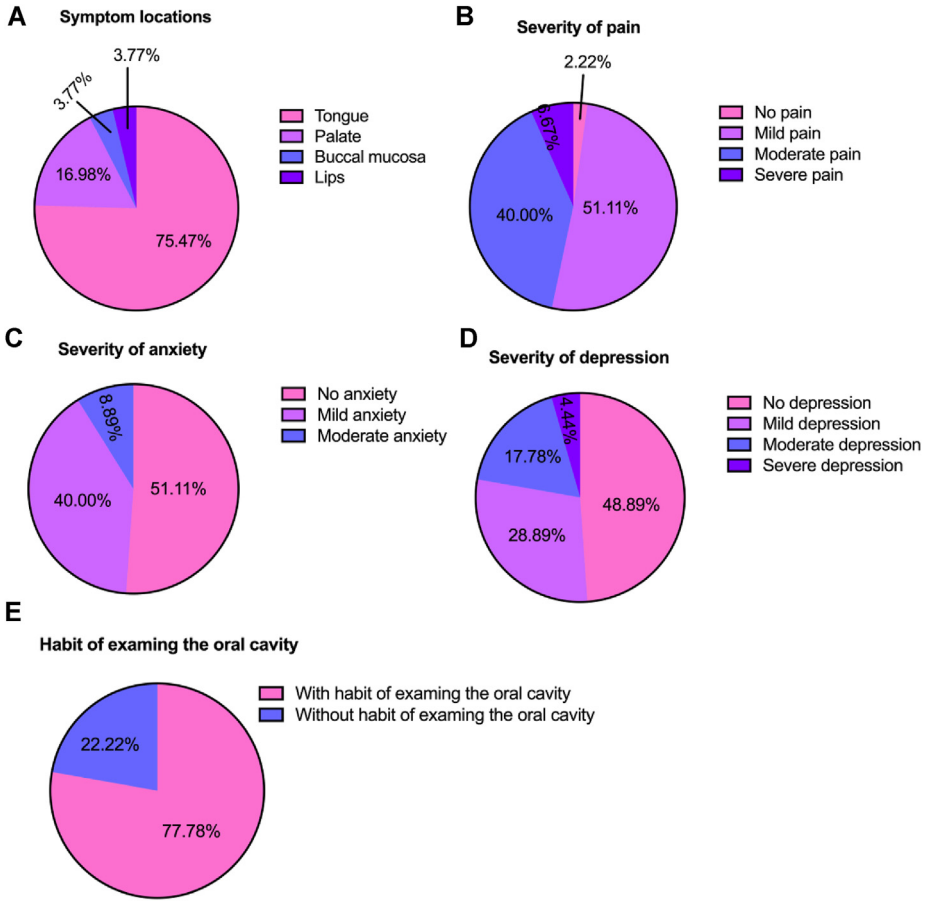


Figure 1 Symptom locations and their related parameters of BMS patients. (A) The proportion of the symptoms occurring on the tongue, palate, buccal mucosa, and lips. The proportion of different degrees of pain (B), anxiety (C), and depression (D). (E) The proportion of people with and without self-examing habits.

Results

Baseline characteristics

In this study, 45 BMS patients and 14 healthy volunteers were recruited. Among the BMS patients, females took up 82.22% while males accounted for 17.78% (Table 1). The symptoms of BMS mainly involved the tongue, followed by the palate, lips, and buccal mucosa (Fig. 1A). Based on VAS scores, we found that 51.11% of patients were experiencing mild pain and 40.00% were undergoing moderate pain (Fig. 1B). To our surprise, only 3 individuals suffered from severe pain, contrary to how BMS patients often describe their disturbing feelings when asking the doctors for help. However, one individual told us that she did not feel pain but suffered from a sensation of numbness on the tongue (Table 2). 77.78% of the individuals troubled by BMS, not all of whom presented with anxiety or depression, were found to have the habit of examining the oral cavity (Fig. 1E).

Anxiety and depression in BMS patients

The 45 BMS patients were divided into groups with anxiety/depression (accounted for 60%) and Groups without anxiety or depression (accounted for 40%) (Table 2). To be more specific, 48.89% of BMS patients were rated as anxious, of whom 40% had mild anxiety, 8.89% had moderate anxiety, and none had severe anxiety (Fig. 1C). Meanwhile, 51.11% of BMS patients were considered as depression, the different severity of which, ranging as mild, moderate and severe, respectively took up 28.89%, 17.78% and 4.44% (Fig. 1D).

Table 1 Democratic characteristics and symptom-related parameters of 45 BMS patients and 14 healthy volunteers.

Variables	Groups	BMS patients (n = 45)	Healthy volunteers (n = 14)
Age (mean ± SD)		55.56 ± 11.40	56.71 ± 13.14
Sex (%)	Female	82.22%	78.57%
	Male	17.78%	21.43%
Symptom locations (%)	Tongue	75.47%	—
	Palate	16.98%	—
	Buccal mucosa	3.77%	—
	Lips	3.77%	—
Habit of examining the oral cavity (%)	With	77.78%	—
	Without	22.22%	—
Severity of pain by VAS (%)	No	2.22%	—
	Mild	51.11%	—
	Moderate	40.00%	—
	Severe	6.67%	—
Severity of anxiety by SAS (%)	No	51.11%	—
	Mild	40.00%	—
	Moderate	8.89%	—
	Severe	0	—
Severity of depression by SDS (%)	No	48.89%	—
	Mild	28.89%	—
	Moderate	17.78%	—
	Severe	4.44%	—

Abbreviations: BMS: burning mouth syndrome; SD: standard deviation; VAS: Visual Analogue Scale; SAS: Zung Self-assessing Anxiety Scale; SDS: Zung Self-assessing Depression Scale.

Comparison of the biomarkers in serum and saliva between BMS patients and healthy volunteers

As is shown in Table 3, among the biomarkers in serum including BDNF, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α, only the BDNF level showed a statistically significant decrease in the patients than the healthy volunteers ($P < 0.05$). However, in terms of BDNF, IL-1β, and IL-8 levels in saliva, a statistically significant increase was found in the BMS patients versus the healthy volunteers ($P = 0.002$, 0.015, and 0.003, respectively). Fig. 2A illustrated a statistically significant difference in BDNF in serum between healthy volunteers and BMS patients either with or without anxiety/depression. Interestingly, INF-γ (in serum) in the two groups of BMS patients was higher than in healthy people, whereas the results of the statistical analysis only showed a significant difference between the patients with anxiety/depression and the healthy volunteers ($P > 0.05$). When it comes to the biomarkers in saliva, BDNF, IFN-γ, and TNF-α in the patients with anxiety/depression exhibited a statistically significant increase versus the healthy people ($P < 0.05$). Moreover, a statistical increase of BDNF, IL-1β, and IL-8 in saliva was found in BMS patients without anxiety or depression than in healthy volunteers ($P < 0.0001$, <0.05, <0.01, respectively) (Fig. 2B).

The associations between VAS, SAS, SDS, and the biomarkers in serum and saliva

Table 4 demonstrated the correlation between the VAS, SAS SDS scores, and the six biomarkers in serum and saliva. There was not a significant correlation between VAS and other parameters ($P > 0.05$), which evidenced that the severity of pain of BMS had little influence on the psychiatric disorders and biomarkers in serum and saliva. In BMS patients with anxiety/depression, a higher level of SAS was negatively related to IL-1β and TNF-α in saliva ($\beta = -0.474$, -0.483, respectively; $P < 0.05$, <0.05, respectively) while a rising level of SDS was negatively associated with BDNF, IL-1β, and IL-8 in saliva ($\beta = -0.418$, -0.515, -0.469, respectively; $P < 0.05$, <0.01, <0.05, respectively). Among the patients without anxiety/depression, except for the correlation between IL-8 in serum and that in saliva, other biomarkers in serum had no association with those in saliva. However, among the patients with anxiety/depression, all the biomarkers in serum showed no relationship with those in saliva. Interestingly, in terms of the inflammatory markers in the saliva of BMS patients with anxiety/depression, TNF-α had strong positive associations with other biomarkers, BDNF, IFN-γ, IL-1β, IL-6, and IL-8 ($\beta = 0.486$, 0.478, 0.559, 0.544, 0.444, respectively; $P = 0.010$, 0.012, 0.002, 0.003, 0.020, respectively) (Fig. 3).

Table 2 Democratic characteristics and symptom-related parameters of BMS patients with anxiety/depression, BMS patients without anxiety/depression, and healthy volunteers.

Variables	Groups	BMS patients with anxiety/depression (n = 27)	BMS patients without anxiety/depression (n = 18)	Healthy volunteers (n = 14)
Age (mean ± SD)		55.11 ± 10.13	56.22 ± 13.05	56.71 ± 13.14
Sex (%)	Female	22 (81.48%)	15 (83.33%)	11 (78.57%)
	Male	5 (18.52%)	3 (16.67%)	3 (21.43%)
Symptom locations (%)	Tongue	23 (85.19%)	17 (94.44%)	—
	Palate	6 (22.22%)	3 (16.67%)	—
	Buccal mucosa	2 (7.41%)	0 (0)	—
	Lips	2 (7.41%)	0 (0)	—
Habit of examining the oral cavity (%)	With	21 (77.78%)	14 (77.78%)	—
	Without	6 (22.22%)	4 (22.22%)	—
Severity of pain by VAS (%)	No	0 (0)	1 (5.56%)	—
	Mild	15 (55.56%)	8 (44.44%)	—
	Moderate	11 (40.74%)	7 (38.89%)	—
	Severe	1 (3.70%)	2 (11.11%)	—
Severity of anxiety by SAS (%)	No	5 (18.52%)	—	—
	Mild	18 (66.67%)	—	—
	Moderate	4 (14.81%)	—	—
	Severe	0 (0)	—	—
Severity of depression by SDS (%)	No	4 (14.81%)	—	—
	Mild	13 (48.15%)	—	—
	Moderate	8 (29.63%)	—	—
	Severe	2 (7.41%)	—	—

Abbreviations: BMS: burning mouth syndrome; SD: standard deviation; VAS: Visual Analogue Scale; SAS: Zung Self-assessing Anxiety Scale; SDS: Zung Self-assessing Depression Scale.

Discussion

BMS is a chronic, debilitating disorder characterized by pain and/or discomfort in the oral cavity. The pathophysiology of BMS is still poorly understood and remains controversial.[17] Notably, Le et al. reported that a majority of individuals infected with BMS (65.6%) presented with an identified psychiatric diagnosis, primarily anxiety and depressive disorders (36.8%).[19] Numerous other studies arrived at similar conclusions regarding the crucial role of

Table 3 Statistical analysis of *P* value between the comparison of salivary and serum biomarkers in BMS patients and healthy volunteers.

		Mean ± SD		*P* value
		BMS patients (n = 45)	Healthy volunteers (n = 14)	
Biomarkers in serum	BDNF	292.95 ± 179.49	576.77 ± 263.94	0.037*
	IFN-γ	7.23 ± 5.40	3.89 ± 3.31	0.342
	IL-1β	4.73 ± 3.18	4.98 ± 7.18	0.262
	IL-6	5.24 ± 4.40	5.91 ± 2.89	0.207
	IL-8	299.79 ± 485.78	503.11 ± 572.20	0.254
	TNF-α	9.33 ± 6.26	5.59 ± 6.61	0.405
Biomarkers in saliva	BDNF	1.97 ± 1.36	0.49 ± 0.54	0.002**
	IFN-γ	4.45 ± 3.98	2.12 ± 2.06	0.088
	IL-1β	403.39 ± 527.00	120.91 ± 160.46	0.015*
	IL-6	8.44 ± 10.09	9.64 ± 19.04	0.326
	IL-8	1104.70 ± 1009.85	385.08 ± 381.12	0.003**
	TNF-α	4.21 ± 2.73	2.87 ± 2.38	0.371

Abbreviations: SD: standard deviation; BMS: burning mouth syndrome; BDNF: brain-derived neurotrophic factor; IFN-γ: interferon-gamma; IL-1β: interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor-alpha.

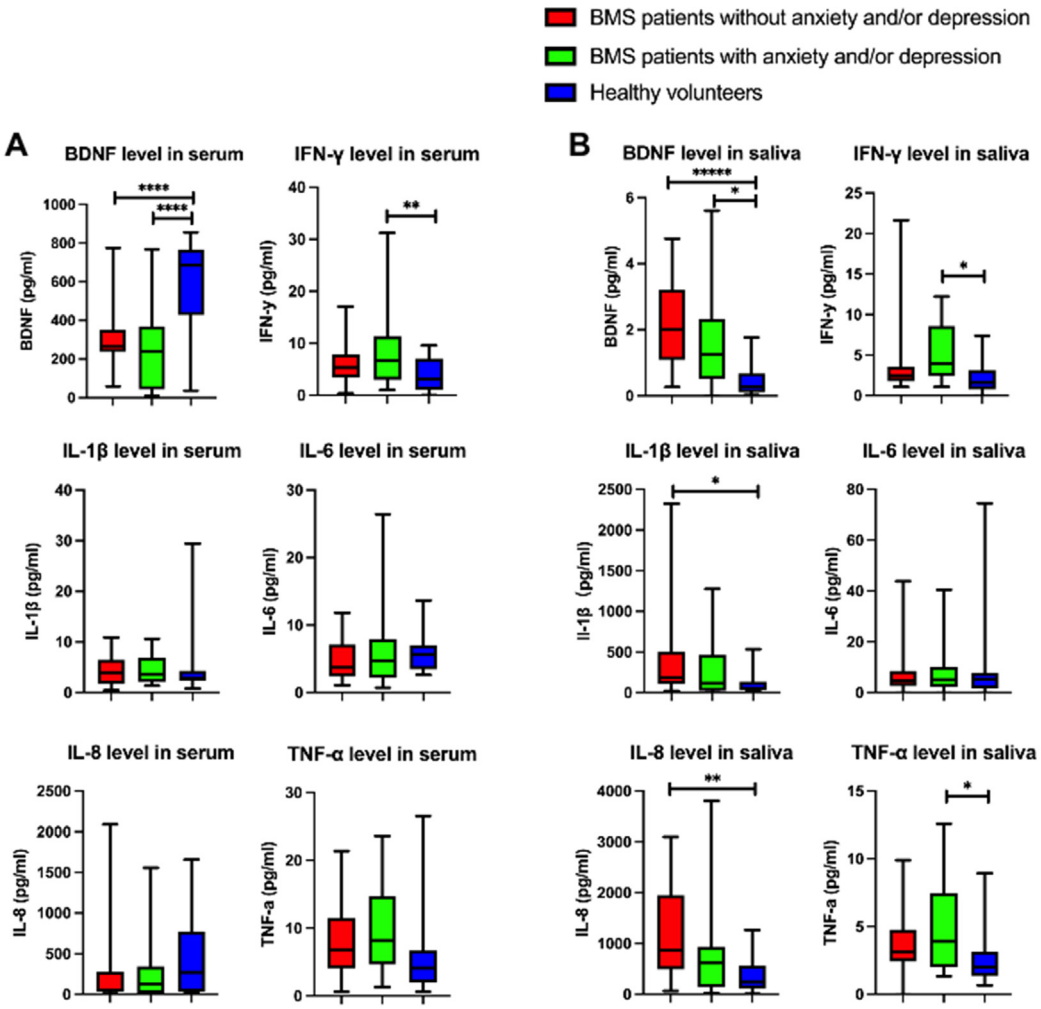


Figure 2 Comparison of the biomarkers in serum and saliva between BMS patients without anxiety/depression, BMS patients with anxiety/depression, and healthy volunteers. (A) The different levels of BDNF, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α in serum among the three groups. (B) The different levels of BDNF, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α in saliva among the three groups. BMS: burning mouth syndrome; BDNF: brain-derived neurotrophic factor; IFN-γ: interferon-gamma; IL-1β: interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor-alpha.

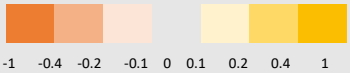
anxiety and depression in BMS.[20–24] However, few of these studies were devoted to the association between mental disorders and saliva/blood in individuals suffering from BMS or the differences between biomarkers in serum and those in saliva. The precise relationship between BMS and anxiety/depression and any predisposition to BMS remains unclear.

BDNF, a protein responsible for synaptic plasticity, is reported to contribute to psychiatric disorders, such as anxiety disorders, depression, schizophrenia, bipolar, etc.[25] IFN-γ and TNF-α, the key molecules controlling pain processes, both show vital interplay in mediating and modulating neuroinflammation, which has been widely demonstrated to be critical in pathological pain.[26] IL-1β, IL-6, and IL-8 are inflammatory cytokines with wide-ranging biological effects. These cytokines have been reported to play a pivotal role in pathological pain (such as peripheral neuropathy, bone cancer, etc.) and psychiatric diseases (such as major depression, anxiety disorders, bipolar disorder, etc).[27–32]

Since the intensity of pain did not correlate with anxiety, depression, or the biomarkers (Table 4), clinicians need to show more empathy and comprehension to all the BMS patients, not only the individuals with anxiety/depression but also the ones without. As Table 4 shows, SAS had a strongly positive correlation with SDS ($\beta = 0.545$, $P < 0.01$), proving the state of anxiety often comes along with depression in BMS patients. The current results reveal the increased level of saliva BDNF, IL-1β, and IL-8 and the decreased level of serum BDNF in BMS patients (whether with anxiety/depression or not) compared to healthy people (Table 3). This finding is consistent with an earlier study by Barry et al. who discovered that plasma IL-8 profiles dysregulated in BMS and another study by André et al. who revealed a significant increase in the IL-1β production from oral mucosa swabs in BMS subjects ($P = 0.005$).[33,34] Many studies explained the pro-nociceptive role of BDNF in pain processes. A previous study by Robert et al. on the elderly with knee osteoarthritis revealed that plasma levels of BDNF decreased after receiving treatment, indicating that

Table 4 Correlations between VAS, SAS, SDS, BDNF, IFN-γ, IL-1β, IL-6, IL-8, and TNF-α in serum and saliva in different groups (spearman correlation coefficients, *P*-values were adjusted for multiple tests).

					Biomarkers in serum						Biomarkers in saliva					
		VAS	SAS	SDS	BDNF	IFN-γ	IL-1β	IL-6	IL-8	TNF-α	BDNF	IFN-γ	IL-1β	IL-6	IL-8	TNF-α
BMS patients with anxiety/depression																
	VAS		0.127	0.248	-0.051	-0.025	-0.013	0.059	-0.341	-0.128	-0.200	0.049	0.056	-0.225	-0.083	-0.076
	SAS			0.545**	-0.138	-0.175	0.242	0.288	0.102	-0.023	-0.364	0.166	-0.474*	-0.140	-0.377	-0.483*
	SDS				0.220	-0.304	-0.109	0.048	-0.008	-0.375	-0.418*	-0.066	-0.515**	0.175	-0.469*	-0.308
	BDNF					-0.276	-0.260	-0.114	0.001	-0.374	-0.066	-0.096	-0.001	0.038	0.035	-0.026
	IFN-γ						0.708**	0.436*	0.094	0.861**	0.100	-0.117	0.111	-0.211	0.083	0.104
Biomarkers in serum	IL-1β							0.819**	0.090	0.797**	-0.152	0.025	-0.039	-.438*	-0.174	-0.241
	IL-6								-0.098	0.507**	-0.036	0.007	-0.009	-0.349	-0.103	-0.306
	IL-8									-0.003	-0.102	-0.023	-0.067	0.159	-0.150	0.014
	TNF-α										0.052	-0.053	0.074	-0.305	0.050	-0.069
	BDNF											-0.013	0.780**	0.206	0.879**	0.486*
	IFN-γ												0.047	0.396*	0.023	0.478*
Biomarkers in saliva	IL-1β													0.091	0.808**	0.559**
	IL-6														0.169	0.544**
	IL-8															0.444*
	TNF-α															
BMS patients without anxiety/depression																
	VAS		0.427	0.086	-0.049	-0.104	-0.021	0.195	0.022	-0.037	-0.256	0.004	-0.320	0.083	-0.436	-0.121
	SAS			0.595**	0.183	-0.465	-0.222	-0.167	-0.123	-0.388	-0.476*	-0.012	-0.151	-0.311	-0.422	-0.075
	SDS				0.300	-0.243	0.040	-0.185	0.038	-0.196	-0.298	-0.245	-0.011	-0.244	-0.003	-0.266
	BDNF					-0.391	0.035	0.040	0.297	-0.172	0.259	-0.560*	0.222	0.092	0.331	-0.540*
	IFN-γ						0.646**	0.500*	0.021	0.681**	0.152	-0.015	0.078	-0.053	0.148	-0.174
Biomarkers in serum	IL-1β							0.822**	0.198	0.840**	0.008	-0.347	0.036	-0.068	0.144	-0.556*
	IL-6								0.461	0.765**	0.068	-0.385	0.101	0.296	0.152	-0.506*
	IL-8									0.274	0.441	-0.324	0.547*	0.429	0.601**	-0.079
	TNF-α										0.208	-0.067	-0.015	0.135	0.164	-0.232
	BDNF											-0.340	0.591**	0.381	0.818**	0.140
	IFN-γ												-0.455	-0.032	-0.542*	0.717**
Biomarkers in saliva	IL-1β													0.254	0.847**	-0.023
	IL-6														0.351	0.105
	IL-8															-0.089
	TNF-α															
Healthy volunteers																
	BDNF					-0.018	0.619*	0.481	0.354	0.018	-0.222	0.109	-0.130	-0.139	-0.101	0.154
	IFN-γ						0.462	-0.095	0.106	0.909**	0.431	0.131	-0.297	-0.501	-0.402	-0.322
Biomarkers in serum	IL-1β							0.529	0.295	0.576*	-0.105	-0.040	-0.387	-0.358	-0.264	-0.174
	IL-6								0.159	-0.093	-0.486	-0.136	-0.442	0.016	-0.139	-0.099
	IL-8									0.121	0.185	0.366	-0.097	-0.130	-0.077	0.218
	TNF-α										0.424	-0.044	-0.064	-0.561*	-0.125	-0.335
	BDNF											0.524	0.409	-0.181	0.284	0.445
	IFN-γ												-0.016	0.292	-0.007	0.680**
Biomarkers in saliva	IL-1β													0.055	0.811**	0.403
	IL-6														0.391	0.535*
	IL-8															0.529
	TNF-α															



*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). Abbreviations: SD: standard deviation; BMS: burning mouth syndrome; BDNF: brain-derived neurotrophic factor; IFN-γ: interferon-gamma; IL-1β: interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor-alpha.

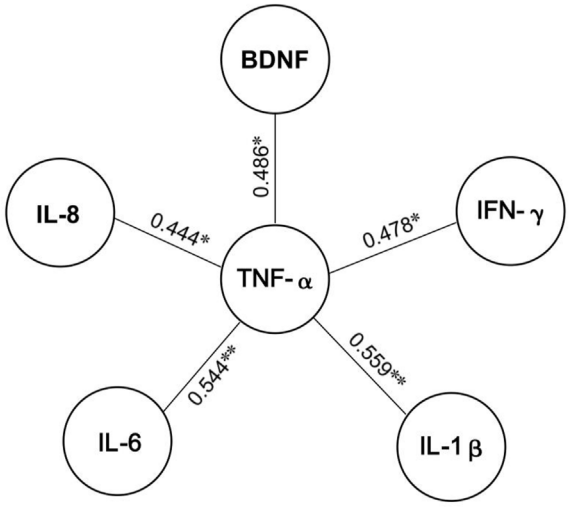


Figure 3 Correlations between salivary TNF-α and other salivary biomarkers (BDNF, IFN-γ, IL-1β, IL-6, and IL-8) among BMS patients with anxiety/depression. TNF-α: tumor necrosis factor-alpha; BDNF: brain-derived neurotrophic factor; IFN-γ: interferon-gamma; IL-1β: interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; BMS: burning mouth syndrome.

the decreased level of BDNF may in turn decrease pain.[35] The current study observed a reduction of serum BDNF in BMS patients, opposite to saliva BDNF. It is reasonable to speculate that the decreased level of serum BDNF contributes to the improvement of pain threshold, making BMS patients better tolerate chronic pain.

When taking anxiety and depression into consideration, in terms of markers in serum and saliva, the patients without anxiety/depression presented the same trends as all the BMS patients as a whole (Table 3 and Fig. 2). However, it is interesting to find a different result in patients with anxiety/depression. Since anxiety and depression had no relationships with markers in serum and only had associations with markers in saliva (as shown in Table 4), it is reasonable to pay more attention to the latter, which showed an increased level of saliva TNF-α, BDNF, IFN-γ among patients with anxiety/depression than healthy volunteers. As illustrated earlier, TNF-α had correlations with the other biomarkers, so it makes sense to speculate that TNF-α might be the upstream factor regulating the other ones. Comparing the healthy volunteers with the patients without anxiety/depression, we find that the latter presented with an increased level of saliva IL-1β and IL-8, which may indicate that anxiety or depression depress the saliva IL-1β and IL-8, so we turn to Table 4 for examination. It is delightful to find that SDS had a negative correlation with the saliva IL-1β and IL-8 while SAS had a negative association with the saliva IL-1β, proving the correctness and logic of our speculation.

In conclusion, the concentration of BDNF in saliva is higher among individuals with BMS than in healthy controls, demonstrating the importance of salivary BDNF level as a diagnostic marker for BMS. Moreover, elevated level of TNF-α in saliva plays a crucial role in BMS patients who also suffer from anxiety/depression, indicating that salivary TNF-α may serve as a promising biomarker for identifying BMS patients with anxiety/depression. Ultimately, the current study holds the potential to advance our comprehension of serum and saliva alterations in BMS patients, as well as their correlations with anxiety/depression status.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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References

1. Ariyawardana A, Chmieliauskaite M, Farag AM, et al. World workshop on oral medicine VII: burning mouth syndrome: a systematic review of disease definitions and diagnostic criteria utilized in randomized clinical trials. *Oral Dis* 2019;25(Suppl 1): 141—56.
2. McMillan R, Forssell H, Buchanan JA, Glenny AM, Weldon JC, Zakrzewska JM. Interventions for treating burning mouth syndrome. *Cochrane Database Syst Rev* 2016;11:CD002779.
3. Klein B, Thoppay JR, De Rossi SS, Ciarrocca K. Burning mouth syndrome. *Dermatol Clin* 2020;38:477—83.
4. Ritchie A, Kramer JM. Recent advances in the etiology and treatment of burning mouth syndrome. *J Dent Res* 2018;97: 1193—9.
5. Russo M, Crafa P, Guglielmetti S, Franzoni L, Fiore W, Di Mario F. Burning mouth syndrome etiology: a narrative review. *J Gastrointestin Liver Dis* 2022;31:223—8.
6. Adamo D, Spagnuolo G. Burning mouth syndrome: an overview and future perspectives. *Int J Environ Res Publ Health* 2022;20: 682.
7. Kolkka-Palomaa M, Jaaskelainen SK, Laine MA, Teerijoki-Oksa T, Sandell M, Forssell H. Pathophysiology of primary burning mouth syndrome with special focus on taste dysfunction: a review. *Oral Dis* 2015;21:937—48.
8. Lee BM, Park JW, Jo JH, Oh B, Chung G. Comparative analysis of the oral microbiome of burning mouth syndrome patients. *J Oral Microbiol* 2022;14:2052632.
9. Feller L, Fourie J, Bouckaert M, Khammissa RAG, Ballyram R, Lemmer J. Burning mouth syndrome: aetiopathogenesis and principles of management. *Pain Res Manag* 2017;2017:1926269.
10. Galli F, Lodi G, Sardella A, Vegni E. Role of psychological factors in burning mouth syndrome: a systematic review and meta-analysis. *Cephalalgia* 2017;37:265—77.
11. Elahi FM, Casaletto KB, La Joie R, et al. Plasma biomarkers of astrocytic and neuronal dysfunction in early- and late-onset Alzheimer's disease. *Alzheimers Dement* 2020;16:681—95.
12. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of salivary biomarkers in oral cancer detection. *Adv Clin Chem* 2018;86:23—70.
13. Liang Y, Lin F, Huang Y. Identification of biomarkers associated with diagnosis of osteoarthritis patients based on

bioinformatics and machine learning. *J Immunol Res* 2022; 2022:5600190.
14. Harsanyi S, Kupcova I, Danisovic L, Klein M. Selected biomarkers of depression: what are the effects of cytokines and inflammation? *Int J Mol Sci* 2022;24:578.
15. Zhou W, Guo J, He M, et al. Fatigue and contributing factors in Chinese patients with ankylosing spondylitis. *Clin Rheumatol* 2020;39:2337—44.
16. Melcer Y, Nimrodi M, Levinsohn-Tavor O, Gal-Kochav M, Pekar-Zlotin M, Maymon R. Analgesic efficacy of intrauterine lidocaine flushing in hysterosalpingo-foam sonography: a double-blind randomized controlled trial. *J Minim Invasive Gynecol* 2021;28:1484—9.
17. Philippot A, Dubois V, Lambrechts K, et al. Impact of physical exercise on depression and anxiety in adolescent inpatients: a randomized controlled trial. *J Affect Disord* 2022;301: 145—53.
18. Liu CY, Yang YZ, Zhang XM, et al. The prevalence and influencing factors in anxiety in medical workers fighting COVID-19 in China: a cross-sectional survey. *Epidemiol Infect* 2020;148: e98.
19. Le Bris V, Chastaing M, Schollhammer M, Brenaut E, Misery L. Usefulness of psychiatric intervention in a joint consultation for the treatment of burning mouth syndrome: a monocentric retrospective study. *Acta Derm Venereol* 2019;99:813—7.
20. Orliaguet M, Misery L. Neuropathic and psychogenic components of burning mouth syndrome: a systematic review. *Biomolecules* 2021;11:1237.
21. Rezazadeh F, Farahmand F, Hosseinpour H, Shahriarirad R, Sabet Eghlidi A. The association between emotional stress, sleep disturbance, depression, and burning mouth syndrome. *BioMed Res Int* 2021;2021:5555316.
22. Sikora M, Verzak Z, Matijevic M, et al. Anxiety and depression scores in patients with burning mouth syndrome. *Psychiatr Danub* 2018;30:466—70.
23. Sevrain M, Brenaut E, Le Toux G, Misery L. Primary burning mouth syndrome: a questionnaire study of neuropathic and psychological components. *Am J Clin Dermatol* 2016;17: 171—8.
24. Tan HL, Smith JG, Hoffmann J, Renton T. A systematic review of treatment for patients with burning mouth syndrome. *Cephalalgia* 2022;42:128—61.
25. Colucci-D'Amato L, Speranza L, Volpicelli F. Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain Cancer. *Int J Mol Sci* 2020;21:7777.
26. Kamieniak P, Bielewicz JM, Grochowski C, et al. IFN-γ correlations with pain assessment, radiological findings, and clinical intercourse in patient after lumbar microdiscectomy: preliminary study. *Dis Markers* 2020;2020:1318930.
27. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: an emerging regulator of pathological pain. *J Neuroinflammation* 2016;13: 141.
28. Zhang B, Chen H, Ouyang J, et al. SQSTM1-dependent autophagic degradation of PKM2 inhibits the production of mature IL1B/IL-1beta and contributes to LIPUS-mediated anti-inflammatory effect. *Autophagy* 2020;16:1262—78.
29. Khan J, Hassun H, Zusman T, Korczeniewska O, Eliav E. Interleukin-8 levels in rat models of nerve damage and neuropathic pain. *Neurosci Lett* 2017;657:106—12.
30. Tsai SJ. Role of interleukin 8 in depression and other psychiatric disorders. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2021;106:110173.
31. Mac Giollabhui N, Foster S, Lowry CA, Mischoulon D, Raison CL, Nyer M. Interleukin-6 receptor antagonists in immunopsychiatry: can they lead to increased interleukin-6 in the central nervous system (CNS) and worsening psychiatric symptoms? *Brain Behav Immun* 2022;103:202—4.
32. Kosuge A, Kunisawa K, Arai S, et al. Heat-sterilized Bifidobacterium breve prevents depression-like behavior and interleukin-1β expression in mice exposed to chronic social defeat stress. *Brain Behav Immun* 2021;96:200—11.
33. Barry A, O'Halloran KD, McKenna JP, McCreary C, Downer EJ. Plasma IL-8 signature correlates with pain and depressive symptomatology in patients with burning mouth syndrome: results from a pilot study. *J Oral Pathol Med* 2018;47:158—65.
34. Campello CP, Pellizzer EP, Vasconcelos B, Moraes SLD, Lemos CAA, Muniz MTC. Evaluation of IL-6 levels and +3954 polymorphism of IL-1β in burning mouth syndrome: a systematic review and meta-analysis. *J Oral Pathol Med* 2020;49: 961—8.
35. Sorkpor SK, Galle K, Teixeira AL, et al. The relationship between plasma BDNF and pain in older adults with knee osteoarthritis. *Biol Res Nurs* 2021;23:629—36.