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## Systematic Review

# Salivary biomarkers and burning mouth syndrome: a systematic review and meta-analysis of the literature

Florian Kappes[1], Samy Kerrad[1], Christelle Grémeau-Richard[1,2], Laurent Devoize[1,2,*]

[1] CHU Clermont-Ferrand, Service d'Odontologie, 63003 Clermont-Ferrand, France
[2] Université Clermont Auvergne, Neuro-Dol, BP 10448, 63000, Clermont-Ferrand & Inserm U1107, 63001 Clermont-Ferrand, France

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**Abstract – Introduction**: Burning Mouth Syndrome (BMS) is defined by a burning sensation or intraoral dysesthesia without obvious causal lesion. Despite the development of research, diagnostic aids for this syndrome are non-existent and treatments are partially ineffective. Some studies have shown changes in salivary composition, viscosity or flow in patients with BMS. The evaluation of the salivary biochemical characteristics of patients with BMS could then help to better understand the pathogenesis of this disease. The objective of this meta-analysis is to perform a qualitative and quantitative synthesis of the literature concerning the salivary biomarkers present in patients with BMS in comparison to healthy subjects. **Materials and methods**: The PubMed, Web of Science and Cochrane databases were searched to identify articles corresponding to the defined inclusion criteria. Review Manager 5.4.1 software was used to perform the quantitative analysis. **Results**: The quantitative analysis included 15 articles and found a significant increase in salivary concentrations of cortisol and immunoglobulin A (IgA) with a standardized mean difference SMD = 0.53 and a 95% confidence interval CI [0.33 to 0.74] and SMD = 0.32 to 95% CI [0.10 to 0.55] respectively. For calcium and copper, the analysis found no significant difference in patients with BMS with SMD = 0.06 at 95% CI [−0.19 to 0.32] and SMD = -0.19 at 95% CI [−0.44 to 0.06]. Finally, for magnesium, the analysis found a probable decrease with SMD = -0.29 at 95% CI [−0.52 to -0.06]. Four other biomarkers (potassium, alpha amylase, zinc and total protein) showed too high levels of heterogeneity ($I^2 > 44\%$) to be able to interpret the results with confidence. **Discussion:** This strong heterogeneity can be explained by different saliva sampling methods or biomarker measurement techniques that are not strictly identical between the studies. In addition, some saliva samples may have undergone blood contamination which may bias the results. **Conclusion:** This meta-analysis nevertheless confirms all the interest of focusing on salivary biomarkers in BMS patients; the measurement of cortisol and/or salivary IgA could be a line of research for the establishment of a standardized biological assessment. Nevertheless, the number of available studies being low and of variable methodological quality with a limited number of patients, additional studies are necessary to give a firm and definitive conclusion.

## Introduction

Burning Mouth Syndrome (BMS) is defined, by the International Classification of Headaches [1], as a burning sensation or intraoral dysesthesia, repeated daily for more than two hours a day for more than three months, without obvious causal lesion. It rather affects postmenopausal women with an estimated prevalence of 14% in this population and a sex ratio of 1 man to 7 women [2,3]. The pain is burnt type and the most common symptoms are altered taste, dysesthesia or xerostomia. This symptomatology most often concerns the anterior two-thirds of the tongue. Thus, the quality of life of patients with this syndrome is heavily degraded. Although the etiopathogenesis of burning mouth syndrome is not clearly identified, BMS is favoured by several factors: local, systemic and psychological [4].

Despite the development of research, the diagnostic means are limited and treatments of this syndrome are not codified. However, some studies have found changes in salivary composition, viscosity or salivary flow in BMS patients [5,6]. The evaluation of the salivary biochemical characteristics of patients with BMS could then make it possible to understand the pathogenesis of this disease and identification of salivary biomarkers could open up new avenues of treatment.

Saliva is a biological fluid, easily accessible and non-invasively, containing a set of biomolecules. It makes it possible to take samples from a wide variety of populations: children, people refractory to blood or urine samples. However,

* Correspondence: laurent.devoize@uca.fr

this biological fluid has some disadvantages that need to be mastered to ensure the reliability of the samples, their quality and their reproducibility. Indeed, its composition varies according to the time of day, hygiene, taking drugs, ...

Although it is composed mainly of water, saliva also contains distinguishable components or biomarkers: namely organic and inorganic elements. These are the concentrations of these biomarkers that we compared in the saliva of BMS patients compared to healthy subjects.

The objective of this work is therefore to carry out a qualitative and quantitative synthesis of the literature concerning salivary biomarkers present in patients with burning mouth syndrome compared to healthy subjects.

## Material and methods

### Search strategy

We conducted a systematic review and a meta-analysis according to the Preferred Reporting items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) statement. The proposed systematic review was registered in PROSPERO under registration number CRD42023403447.

A bibliographic research was conducted in the following databases: PubMed, Web of Science and The Cochrane Library. The following keywords were used for the search strategy: biomarker, saliva, burning mouth syndrome.

All reference lists of previously selected studies were manually reviewed to identify articles on salivary biomarker concentration in patients with burning mouth syndrome compared to a cohort of healthy subjects. Two examiners carried out the bibliographic search in parallel and compared the selected articles.

The search included literature published prior to September 2022.

### Selection of studies and eligibility of criteria

- Inclusion criteria

All types of articles published in English or French that examined salivary biomarker concentrations in patients with burning mouth syndrome compared to healthy subjects were included.

The diagnostic criteria for burning mouth syndrome were established, for each patient, on the basis of the following clinical results: present symptoms of continuous oral burns, for at least two hours a day and this for more than three months.

- Exclusion criteria

The following were excluded:
- Studies carried out in animals or in humans *in vitro*.
- Studies in which salivary biomarkers have not been performed in both healthy patients and patients with burning mouth syndrome.
- Studies published in a language other than English and French.
- Studies where diagnostic criteria for burning mouth syndrome are not explained.
- Studies with application of an ongoing treatment.
- Studies whose results have not been published or where full texts are not available.

The factors that have been excluded are:
- Objectivable oral lesion(s).
- Blood-abnormalities that can explain the symptoms (vitamin B12 deficiency, folate, iron, ...).
- Oral pain that can be attributed to other pathologies (oral lichen, candidiasis, gastroesophageal reflux, ...).
- Known renal or hepatic cancers or dysfunctions.
- Oral pain other than burning mouth syndrome such as Sjögren's syndrome or latent infection.
- Treatments that can interfere with the study and induce a xerostomia: antihistamines, anti-depressants, anti-hypertensives, ...

### Data collection

The studies identified during the electronic search stages were listed in a summary table specifying for each article: the name of the lead author, the year of publication, the title, the type and design of the study, the type of quantification, the number of BMS patients and healthy subjects, and the age of the patients and healthy subjects.

After this identification phase, a flowchart was drawn up graphically representing the study selection process.

### Risk of bias in the evaluation

Case selection was considered adequate when patients were representative of the defined pathology, *i.e.* burning mouth syndrome.

The selection of controls was appropriate when they were sampled from the same population as the cases. Comparability between cases and controls was assessed by sex and age.

### Group definition

In case–control studies, comparisons were divided into two categories: the concentration of the biomarker found in healthy people and the concentration of the biomarker found in patients with burning mouth syndrome. Healthy subjects had to be free of clinical signs of BMS and not have significant systemic pathology (cancer, Biermer's anemia, ...) or treatment that could interfere with the study (immunosuppressive treatment, anti-inflammatory, ...).

According to the articles, the determination of salivary biomarkers was performed in stimulated or unstimulated saliva and in total or specific gland saliva.

### Quantitative analysis

Quantitative data on salivary biomarker assays from BMS and control patients were extracted to calculate, for each study, the standardized mean difference (SMD) and the 95% confidence interval (95% CI). All data were reported in summary tables. Statistical measurements were performed with Review Manager 5.4.1 software.

Standardized mean difference (SMD) is used as a statistical tool in meta-analysis when all selected studies assess the same outcome but measure it in different ways. In these circumstances, it is necessary to standardize the results of the studies on a uniform scale before they can be grouped. The standardized mean difference expresses the size of the intervention effect in each study compared to the variability observed in that study.

$$\text{SMD} = \frac{\text{difference in meanscore between groups}}{\text{the standard deviation of the results between the participants}}.$$

Thus, studies for which the difference in mean between groups is the same proportion as the standard deviation between participants, will have the same SMD, regardless of the actual scales used to perform the measurements.

Necessarily, the studies selected for a systematic review of the literature will not be perfectly identical and all forms of variability within these studies will be grouped under the generic term heterogeneity. Clinical variability consists of differences observed within the recruited population, the nature of the intervention performed, or outcomes. Methodological variability concerns the risk of bias and the design of each study. The variability in effect measurement across studies is the result of the sum of clinical and methodological variability and can be quantified by measuring statistical heterogeneity.

The $Chi^2$ test, proposed by Review Manager, assesses whether the observed difference between studies is due to hazard alone. A low $p$ or high $Chi^2$ value indicates heterogeneity in estimating the effect of an intervention. The interpretation of these figures should be cautious as it is highly dependent on the number of studies selected and the sample size of each. Studies with a small number of subjects or a small number of studies expose heterogeneity to under screening. Therefore, while a statistically significant result may indicate a problem of heterogeneity, a non-significant result should not be considered evidence of the absence of heterogeneity. This is also the reason why a $P$-value of 0.10 is sometimes used rather than the conventional level of 0.05 to determine statistical significance.

Thus, another method, the measurement of inconsistency, was developed to measure, not the existence of heterogeneity, but its impact on the result of the meta-analysis.

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%.$$

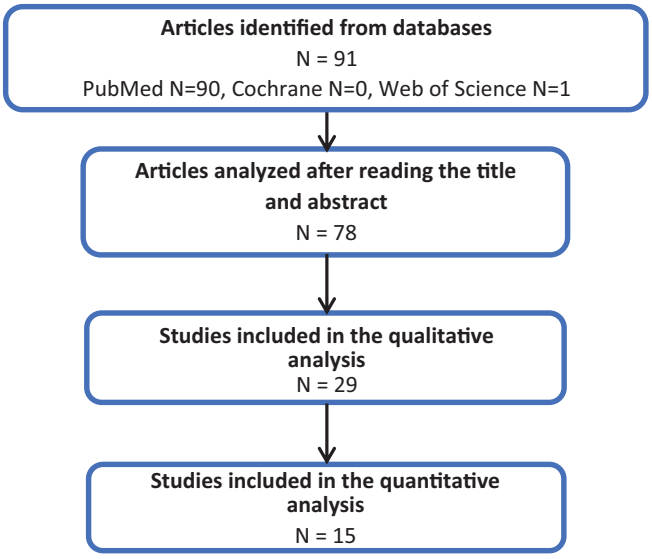



**Fig. 1.** Flowchart representing the bibliographic selection strategy

$Q$ = $Chi^2$ test result and df = number of degrees of freedom of $Chi^2$, $I^2$ represents the percentage of variability in the measure of effect due to heterogeneity rather than sampling fluctuations.

The value of $I^2$, according to the Cochrane Handbook for Systematic Review, is interpreted as follows:
- 0% to 40%: Low heterogeneity.
- 30% to 60%: Moderately significant heterogeneity.
- 50% to 90%: Heterogeneity to be considered.
- 75% to 100%: Very high heterogeneity.

## Results

### Study selection and characteristics

In total, the search equation led to an initial selection of 91 articles, 78 of which were eligible after reading the title and abstract. After complete reading of the articles, 29 articles were included in the qualitative analysis and 15 in the quantitative analysis. The selection of studies is represented in the flowchart (Fig. 1).

All characteristics of the 29 studies included in the qualitative analysis were grouped in a table format (Tab. I). It brings together studies of salivary biomarkers in BMS patients compared to healthy controls. Not all of these studies were included in the quantitative analysis and 15 articles were used to conduct a meta-analysis. All the results of the quantitative analysis of the 15 studies were grouped in tabular form (Tab. II).

### Description of studies eligible for quantitative analysis

The 15 papers selected for quantitative analysis enrolled a population of 620 patients and 474 healthy subjects. Two articles were cross-sectional studies [7,8] and the remainder were case-control studies. Six studies included only women

**Table I.** Summary of study characteristics and assessment of risk of bias of studies.

| Study characteristics | Studies *n* (%) | Assessment of bias | Studies *n* (%) |
|---|---|---|---|
| **Year of publication** | | **Selection** | Adequate |
| <2005 | 3 (10%) | Definition of cases | 26 (90%) |
| 2005–2010 | 11 (38%) | Selection of cases | 26 (90%) |
| 2011–2014 | 4 (13%) | Definition of controls | 25 (86%) |
| ≥2015 | 11 (38%) | Selection of controls | 25 (86%) |
| **Study design** | | **Comparabiliy** | |
| Control cases | 25 (86%) | In age and sex | 23 (79%) |
| Cross sectional study | 4 (14%) | Other factors | 13 (44%) |
| **Types of cases** | | **Measures** | |
| BMS | 29 (100%) | Description of measures | 29 (100%) |
| **Types of healthy subjects** | | Validation of the technique | 29 (100%) |
| Healthy | 29 (100%) | | |

[6,9–12]. Age intervals were given by De Moura *et al.* [13] (BMS and control: 45–84 years). No information on patient age or controls was given in Acharya *et al.* [11].

Aitken *et al.*, Amenabar *et al.*, Kim *et al.* and Nosratzehi *et al.* [9,10,14,15] transcribed salivary cortisol concentrations. Alpha amylase was identified in five papers [6,7,9,10,16], calcium in three [8,13,17], copper in three [8,17,18], magnesium in four [8,13,17,18]; but also potassium in three [8,13,16], zinc in three [8,17,18], immunoglobulin A in four [6,7,11,16], and finally total proteins in five [11,13,16,19,20].

In the majority of studies, quantification of salivary compounds was performed by the enzyme-linked immunosorbent assay ELISA.

According to studies, the clinical diagnosis of burning mouth syndrome has been defined in different ways. In many cases, an inspection of the mucous membranes is carried out as well as a medical anamnesis: burning sensation for more than 6 months, blood examination, exclusion of pathology that may induce oral symptoms [7,8,10,19].

## Analysis of risk of bias in studies

### Power bias

The numbers of each population in the different studies are never very large and range from 8 to 180 subjects per patient group. Most studies suggest using larger numbers.

### Sampling bias

Based on available population descriptions, all fifteen studies recruited cases and controls from the same population.

Most salivary biomarker studies applied adequate diagnostic criteria.

Sampling and measurement methods were adequate and described in all studies.

The main technique for analysing the concentration of salivary biomarkers was done with the ELISA test in almost all studies.

### Heterogeneity bias

The main bias of our study is represented by the heterogeneity of the samples that were carried out differently: technique, type and level of saliva stimulation. We therefore chose to analyse the normalized mean difference (SMD) rather than the relative risk (RR), in order to correct this bias. We also ensure consistency in the fifteen final studies selected with regard to study populations and inclusion/exclusion criteria.

## Quantitative analysis

We conducted several rounds of benchmarking analysis. The results of each analysis (SMD, 95% CI) are presented using Review Manager 5.4.1.

The units of the biomarkers concerned were standardized after being collected from healthy subjects and BMS patients
- In IU/mL for alpha amylase.
- In ng/mL for cortisol.
- In mg/dL for calcium, magnesium, immunoglobulin A, total proteins.
- In ug/L for copper, zinc.
- In mg/L for potassium.

Seventy-four compounds were identified in saliva (Tab. III). Biomarkers that appear in more than two studies are: alpha amylase, cortisol, calcium, magnesium, immunoglobulin A, total protein, copper, zinc and potassium. Shigeyama *et al.* and de Souza *et al.* did not give mean and standard deviation values for cortisol [24,29]. Glick *et al.* does not give a value for calcium [31]. Henkin *et al.*, Borelli *et al.* and Hershkovitch *et al.* do not indicate the standard deviation but the SEM [16,17,19].

**Table II.** Characteristics of selected studies.

| | Type of study | Sex | Number of BMS | Number of controls | Mean age of subjects ± SD (min–max) | Type of quantification |
|---|---|---|---|---|---|---|
| Lopez-Jornet *et al.*, 2020 | Cross sectional | BMS : 45W/6M Contrl : 26W/5M | 51 | 31 | Control : 58.3 ± 11.05 BMS : 59.12 ± 12.29 | Total protein and uric acid: colorimetric kit for urine<br>Total IgA: ELISA<br>alpha amylase: commercial colorimetric kit |
| Lopez-Jornet *et al.*, 2014 | Cross sectional | Control 13M and 17W<br>BMS 6M and 22W | 28 | 30 | Control 50 ± 17.2<br>BMS 58.8 ± 10.2 | Spectrometry |
| Imura *et al.*, 2016 | Case–control | Only women | 15 | 30 | BMS 55.20 ± 6.05<br>Control 52.27 ± 3.49 | IgA : ELISA |
| Nosratzehi *et al.*, 2017 | Case–control | Only women | 30 | 30 | BMS: 39.6 ± 13.3 Control : 39.5 ± 13.2 | Cortisol : ELISA<br>Amylase : spectrophotometry |
| Amenabar *et al.*, 2008 | Case–control | MS: 24W/6M, Control: ? | 30 | 30 | BMS: 61.6 ±10.7, Control : 63.7 ± 9.7 | Radio immunoassay |
| De Moura *et al.*, 2007 | Case–control | Each group 23W 1M | 24 | 24 | For all patients (45–84) | ELISA |
| Aitken-Saavedra *et al.*, 2021 | Case–control | Only women | 40 | 40 | BMS: 62.7 ± 10.8 (37–84), Control: 48.5 ± 12.35 (30–66) | ELISA |
| Kim *et al.*, 2012 | Case–control | Only women | 15 | 14 | BMS: 54.5 ± 6.3 Control: 70.6 ± 5.7 | ELISA |
| Suh *et al.*, 2009 | Case–control | Only women | 40 | 20 | BMS: 61.6 ± 10,1 Control: 65.1 ± 9.0 | ELISA |
| Acharya *et al.*, 2019 | Case–control | Only women | 8 | 8 | ? | ELISA |
| Borelli *et al.*, 2010 | Case–control | BMS 17 W 3M<br>Control 16 W 3 M | 20 | 20 | BMS : 69 (41–89),<br>Control : 68–3 (41–77) | ELISA (NGF / SP / Calprotectin)<br>spectrophotometry (Tryptase / MPO)<br>Bradford method proteins |
| Hershkovich *et al.*, 2004 | Case–control | BMS 120 W 60 M<br>Control : 62 W 28 M | 180 | 90 | BMS : 58 ± 11,<br>Control : 57 ± 16 | (K) + (Na): spectrophotometry<br>Total protein: Lowry method<br>Amylase: Phadebas test<br>Lysozyme, IgM, IgG, IgA, and albumin radial immunodiffusion method |
| Boras *et al.*, 2010 | Case–control | Only women | 26 | 22 | | Total protein and uric acid: colorimetric kit for urine |

**Table II.** (continued).

| | Type of study | Sex | NumberNumber of BMS of controls | | Mean age of subjects ± SD (min–max) | Type of quantification |
|---|---|---|---|---|---|---|
| | | | | | BMS: 65.69 (57–78), Control : 49.72 (24–82) | Total IgA: ELISA alpha amylase: commercial colorimetric kit |
| Pekiner *et al.*, 2009 | Case–control | BMS 19 W 11 M Control 21 W 9M | 30 | 30 | BMS: 54.23 ± 12.78 Control: 50,87 ± 6,23 | Cu, Mg and Zn: atomic absorbance spectrophotometry Interleukins: ELISA |
| Henkin *et al.*, 2012 | Case–control | BMS : 61 W 22 M Control : 43 W 12 M | 83 | 55 | BMS: 60 ± 4 Control: 49 ± 6 | Spectrophotometry |

For calcium, statistical analysis gives us a SMD = 0.06 CI [−0.19 to 0.32]. Saliva calcium concentrations in BMS patients were unchanged compared to healthy subjects ($P > 0.05$). The very low heterogeneity found ($I^2 = 0\%$) confirms the result, which we can confirm for sure (Fig. 2A).

For magnesium, statistical analysis gives us a SMD = −0.29 [−0.52 to −0.06]. Magnesium concentrations in saliva in BMS patients were decreased compared to healthy subjects ($P < 0.05$). The low heterogeneity found ($I^2 = 37\%$) confirms the result. This decrease in salivary magnesium in BMS patients compared to controls is a statistically relevant (Fig. 2B).

For cortisol, statistical analysis gives us a SMD = 0.53 [0.33 to 0.74]. Saliva cortisol concentrations in BMS patients were increased compared to healthy subjects ($P < 0.05$). The very low heterogeneity found ($I^2 = 0\%$) confirms the result. This increase in salivary cortisol in BMS patients compared to controls is a statistical data that we can say almost certainly (Fig. 2C).

For potassium, statistical analysis gives us a SMD = 0.06 [−0.17 to 0.30]. Saliva potassium concentrations in BMS patients were unchanged compared to healthy subjects ($P < 0.05$). The considerable heterogeneity found ($I^2$=62%) does not allow us to give a sure conclusion (Fig. 2D).

For amylase, statistical analysis gives us a SMD = 0.67 [0.47 to 0.87]. Concentrations of alpha amylase in saliva in BMS patients were increased compared to healthy subjects ($P < 0.05$). The considerable heterogeneity found ($I^2 = 59\%$) does not allow us to give a certain conclusion (Fig. 2E).

For zinc, statistical analysis gives us a SMD = 0.13 [−0.12 to 0.38]. Saliva zinc concentrations in BMS patients were unchanged compared to healthy subjects ($P > 0.05$). The significant heterogeneity found ($I^2 = 44\%$) does not allow us to give a sure conclusion (Fig. 2F).

For immunoglobulin A, statistical analysis gives us a SMD = 0.32 [0.10 to 0.55]. Concentrations of immunoglobulin A in saliva in BMS patients were increased compared to healthy subjects ($P < 0.05$). The very low heterogeneity found ($I^2 = 0\%$) confirms the result. This increase in salivary immunoglobulin A in BMS patients compared to controls is a statistical data that we can say almost certainly (Fig. 2G).

For total proteins, statistical analysis gives us a SMD = 0.29 [0.09 to 0.50]. Total protein concentrations in saliva in BMS patients were increased compared to healthy subjects ($P < 0.05$). The considerable heterogeneity found ($I^2 = 65\%$) does not allow us to give a sure conclusion (Fig. 2H).

For copper, statistical analysis gives us a SMD = −0.19 [−0.44 to 0.06]. Copper concentrations in saliva in BMS patients were unchanged compared to healthy subjects ($P > 0.05$). The very small heterogeneity found ($I^2 = 0\%$) confirms the result, which we can say with certainty (Fig. 2I).

## Discussion

This systematic review and meta-analysis included papers that analysed salivary concentrations of different biomarkers in patients with burning mouth syndrome, comparing their results

**Table III.** Summary of salivary biomarker measurements in BMS patients.

| | Studies N= | BMS |
|---|---|---|
| **Neurotransmitters** | | |
| Glutamatergic system | | |
| Glucose | 1 | = [13] |
| Serotonergic system | | |
| Tryptase | 1 | ↑ [19] |
| **Neuropeptides** | | |
| Endogenous opioids | | |
| Opiorphin | 2 | UWS ↑ [21], SWS = [21], = [22] |
| Tachykines | | |
| Substance P | 2 | ↓ [19], = [12] |
| Neurokinin A | 1 | = [12] |
| Other neuropeptides | | |
| Calcitonin gene-related peptide (CGRP) | 2 | = [12], = [23] |
| **Neurotrophins** | | |
| Brain-derives neurotrophic factor | 1 | = [24] |
| Nerve growth factor (NGF) | 2 | = [24], ↑ [19] |
| **Enzymes** | | |
| Alpha-amylase | 5 | ↑ [6], ↑ [9], ↑ [10], ↑ [16], ↑ [7] |
| Alpha-enolase | 1 | ↑ [25] |
| Cathepsin G | 1 | = [25] |
| Lysozyme | 1 | ↑ [16] |
| Myeloperoxydase | 1 | = [19] |
| **Cytokines** | | |
| Tumor necrosis factor alpha | 2 | ↓ [24], = [20] |
| EGF | 1 | = [11] |
| VEGF | 1 | = [11] |
| Interleukin 2 | 2 | ↑ [26], = [18] |
| Interleukin 6 | 4 | ↑ [24], = [20], ↑ [26], = [18] |
| Interleukin 8 | 3 | ↑ UWS [27], = [20], = [11] |
| Interleukin 10 | 1 | = [24] |
| Interleukin 1 | 1 | = [20] |
| Macrophage Inflammatory Protein-4 (MIP4) | 1 | ↑ [7] |
| Interleukin 18 | 1 | ↑ [25] |
| **Steroid hormones** | | |
| 17-Estradiol | 2 | = UWS, ↑ SWS [10], ↓ [28] |
| Cortisol | 6 | = [9], ↑ [15], = [24], ↑ UWS SWS [10], ↑ [14], ↑ [29] |
| DHEAs | 3 | = [10], ↓ morning = night [30], = [28] |
| Progesteron | 2 | = [10], = [28] |
| **Metallic ions** | | |
| Aluminium | 1 | = [8] |
| Boron | 1 | = [8] |
| Calcium | 4 | = [8], = [13], = [17], = [31] |
| Chlorine | 1 | ↑ [13] |
| Copper | 3 | = [8], = [18], = [17] |
| Iron | 2 | = [8], = [13] |
| Magnesium | 5 | = [8], = [13], ↓ [18], = [17], = [31] |
| Manganese | 1 | = [8] |
| Phosphorus | 3 | = [8], ↑ [13], ↑ [31] |

**Table III.** (continued).

| | Studies N= | BMS |
|---|---|---|
| Potassium | 4 | = [8], ↑ [13], = [16], ↑ [31] |
| Sulfur | 1 | = [8] |
| Zinc | 3 | = [8], = [18], = [17] |
| Sodium | 3 | = [8], ↑ [16], = [31] |
| Cadmium | 1 | = [8] |
| Chromium | 1 | = [8] |
| Arsenic | 1 | = [8] |
| Beryllium | 1 | = [8] |
| Bismuth | 1 | = [8] |
| Cobalt | 1 | = [8] |
| Lithium | 1 | = [8] |
| Molybdenum | 1 | = [8] |
| Antimony | 1 | = [8] |
| Selenium | 1 | = [8] |
| Strontium | 1 | = [8] |
| Titanium | 1 | = [8] |
| Thallium | 1 | = [8] |
| Vanadium | 1 | = [8] |
| Nickel | 1 | = [8] |
| Lead | 1 | = [8] |
| **Others** | | |
| Uric acid | 3 | ↓ [7], = [13], = [16] |
| Albumin | 1 | ↑ [16] |
| Calprotectin | 1 | = [19] |
| Chondroitin Sulfate | 1 | ↓ [32] |
| Chromogranin A | 1 | ↑ [29] |
| Immunoglobulin A | 4 | ↑ [7], = [6], = [11], ↑ [16] |
| Immunoglobulin G | 1 | ↑ [16] |
| Immunoglobulin M | 1 | ↑ [16] |
| Kallikrein | 2 | ↑ [25], ↑ [32] |
| Nitric products (NO) | 1 | = [33] |
| Thiocyanate | 1 | = [13] |
| Total protein | 7 | = [13], = [20], = [11], = [19], ↑ [16], ↓ SWS woman and = man [34], ↑ [31] |
| ROS | 1 | ↓ [33] |
| Nitrite | 1 | = [33] |
| Nitrate | 1 | = [33] |
| IFN-$\gamma$ | 1 | ↑ UWS [27] |
| Neutrophil myeloperoxidase MPO | 1 | = [19] |
| Sialic acid | 1 | = SWS [34] |

Biochemical compounds found in the literature in BMS patients compared to control patients. ↑ means significantly increased concentration, ↓ means significantly decreased concentration, = means similar concentrations, UWS stands for unstimulated saliva, SWS stands for stimulated saliva. [1]Article number (see bibliography).

to a control group. A total of 620 patients were studied. The prevalence of the disease in women, especially after menopause, is indicated by several studies [35–37]. And identifying biomarkers whose salivary concentration is changed during the presence of the syndrome could facilitate the diagnosis of this pathology.

There is already a systematic review and meta-analysis in the literature. However, this one found only 54 different biomarkers against 74 for ours. Moreover, in this study, only 3 biomarkers (cortisol, $\alpha$-amylase, and dehydroepiandrosterone) were found in 3 or more studies whereas there are 9 biomarkers in ours.

For BMS patients, the meta-analysis does not find any difference in variations in the concentration of calcium or copper, but an increase in cortisol and immunoglobulin A, which can be confirmed formally. Cortisol is a glucocorticoid secreted by the adrenal gland and it is a biomarker associated with stress. This meta-analysis found higher levels of this salivary biomarker in BMS patients. It is in agreement with previous studies that reflect how anxiety, depression, and stress levels are frequently associated with BMS [7]. So, it would be interesting to evaluate whether therapies for BMS reduce cortisol levels in these patients, as the determination of cortisol in saliva could be a reliable biomarker to evaluate the response to treatment.

IgA is a salivary glycoprotein with immunological function that acts as a defense against pathogens. This meta-analysis found higher levels of this salivary biomarker in BMS patients. It is not in agreement with the theory that this first line of defense is altered in BMS patients, as other studies had hypothesized [6].

For magnesium we find a decrease in its salivary concentrations in BMS patients in a relevant way.

For zinc and potassium, we do not observe any difference in concentration with statistical results which do not confirm this.

For alpha amylase and total proteins, we find an increase in their concentrations but without relevant statistical results.

We then find a significant variability in our analyses which is observed by a significant heterogeneity in certain cases, such as for alpha amylase ($I^2 = 59\%$) or potassium ($I^2 = 62\%$). This may be explained by different saliva sampling methods or non-identical biomarker measurement techniques between studies.

During the salivary collection, it is then necessary to take into account the type of saliva (total or specific of a gland) and the level of stimulation (stimulated or unstimulated).

According to Navazesh [38], there are different methods for collecting unstimulated total saliva. Five minutes is an adequate collection time and the four most common methods are:

- Draining method: the patient lowers the head and lets saliva flow along the lower lip into a graduated test tube equipped with a funnel.
- The spitting method: the patient spits out the saliva that accumulates in a graduated test tube every sixty seconds.
- The suction method: saliva is continuously aspirated from the floor of the mouth into a test tube for suction.
- The swab method: saliva is collected by a pre-weighed swab, a cotton roll or a gauze sponge placed in the mouth at the orifices of the main salivary glands and is removed to be weighed again at the end of the collection period.

With regard to stimulated saliva, chewing paraffin wax, the use of citric acid or mechanical stimuli allow the stimulation of the glands [39].

Lopez-Jornet *et al.* [7] uses the draining method for 5 minutes then the saliva is centrifuged for 10 min then the aliquot of the supernatants is stored at −80 °C. Nosratzehi *et al.* [9] on the other hand uses the spitting method but centrifuges for 20 min and stores at −20 °C. Finally for de Moura *et al.* [13], saliva is also collected by the spitting method, centrifuged for 10 min but there is no information on the storage temperature of the aliquots. Hershkovitch *et al.* [16] also uses the spitting method for 5 min, the samples are centrifuged without duration and then stored at 4 °C.

In addition, the methodology for analysing the compounds chosen varies according to the biomarker and according to the studies.

Hershkovitch *et al.* [16] uses the Phadebas test to measure salivary concentrations of alpha amylase, while Lopez-Jornet *et al.* [7] uses a colorimetric kit and Nosratzehi *et al.* [9] a spectrophotometer.

For salivary immunoglobulin A concentrations, Hershkovitch *et al.* [16] uses Macini's method while Acharya *et al.*, Imura *et al.* and Lopez-Jornet *et al.* [6,7,11] use the Elisa test.

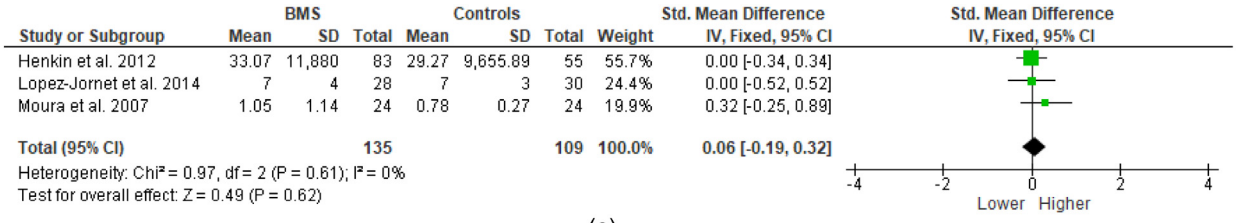
For salivary cortisol concentrations Nosratzehi *et al.*, Kim *et al.* [9,10] use the Elisa Aitken method [14] as well.

Finally, the sample sizes of the different populations range from 8 for Acharya *et al.* [11] to 180 for Herskovitch *et al.* [16] per group. This is one of the limits mentioned by each of the articles: the workforce must be increased.

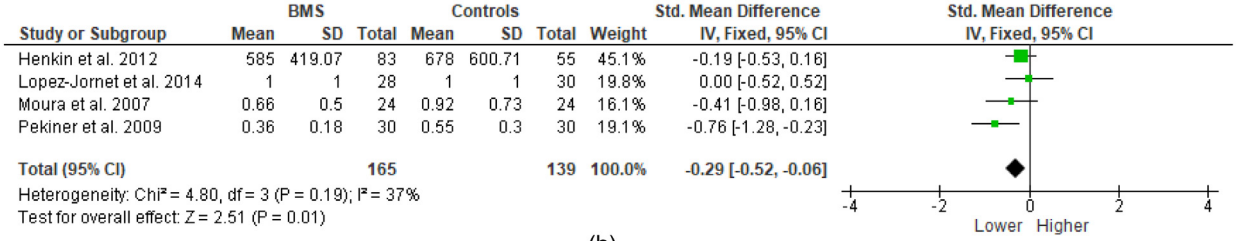
In addition, some saliva samples may have undergone blood contamination which may bias the results. Indeed, for example, the total number of proteins in plasma is 10–100 times higher in blood than in saliva [40]. However, not all compounds are influenced by the presence of blood in the saliva. Some proteins are produced specifically by the salivary glands and are present only in saliva and not in the blood. [41]. Some studies perform a visual examination of the samples [7], others measure transferrin in salivary samples [10] while others do not provide any precision [19]. It could be interesting to measure the salivary levels of haemoglobin, albumin, and transferrin [41].

Some studies [6,9,11] are only carried out in populations of women while others [7,17,18] in populations of men and women, which could explain the differences in salivary concentrations for a same compound.

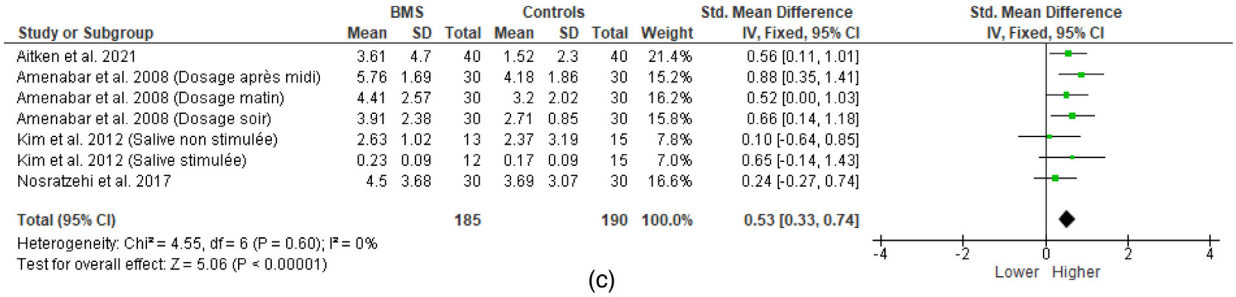
Finally, not all studies are based on the same diagnostic criteria: Borelli *et al.* and Acharya *et al.* and Aitken *et al.* [11,14,19] rely on those of the International Headache Society while Nosratzehi *et al.* [9] relies on those of Scala *et al.* [4], which shows the difficulty of carrying out the diagnosis of this pathology. The methodological quality of the studies being variable with a limited number of patients, additional studies are necessary to give a firm and definitive conclusion.



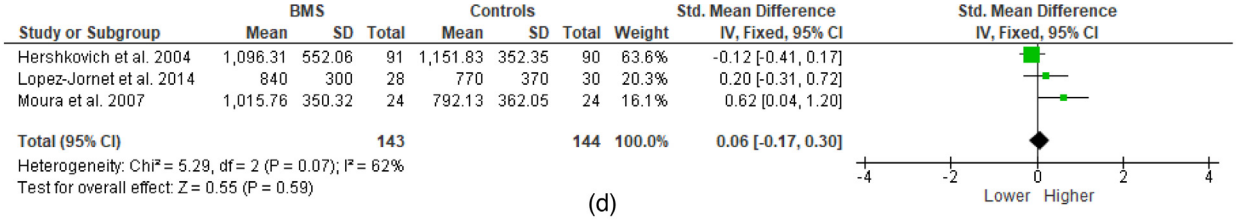

(a)



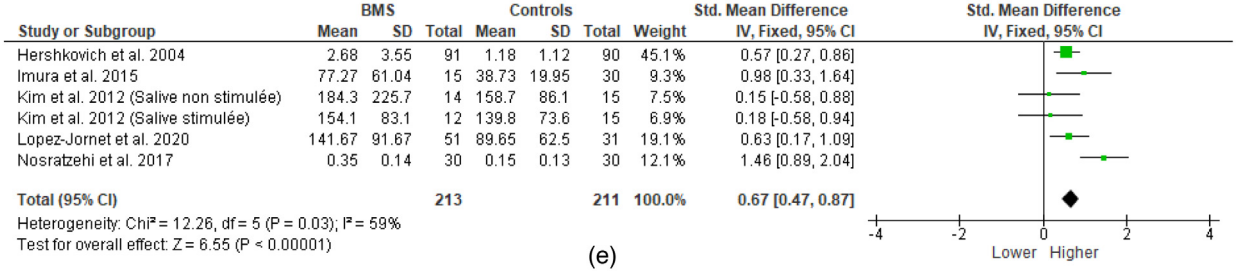

(b)




(c)




(d)




(e)

**Fig. 2.** (A) Forest plot of calcium in BMS patients. (B) Forest plot of magnesium in BMS patients. (C) Forest plot cortisol in BMS patients. (D) Forest plot of potassium in BMS patients. (E) Forest plot of alpha amylase in BMS patients. (F) Forest plot of zinc in BMS patients. (G) Forest plot of immunoglobulin A in BMS patients. (H) Forest plot of total proteins in BMS patients. (I) Forest plot of copper in BMS patients. The squares represent the effect sizes of each of the studies (the size is proportional to the weight of the study) and the lines on the abscissa indicate the 95% confidence interval. The solid diamonds represent the size of the overall effect (on the abscissa the width indicates the 95% confidence interval). Total: number of subjects.

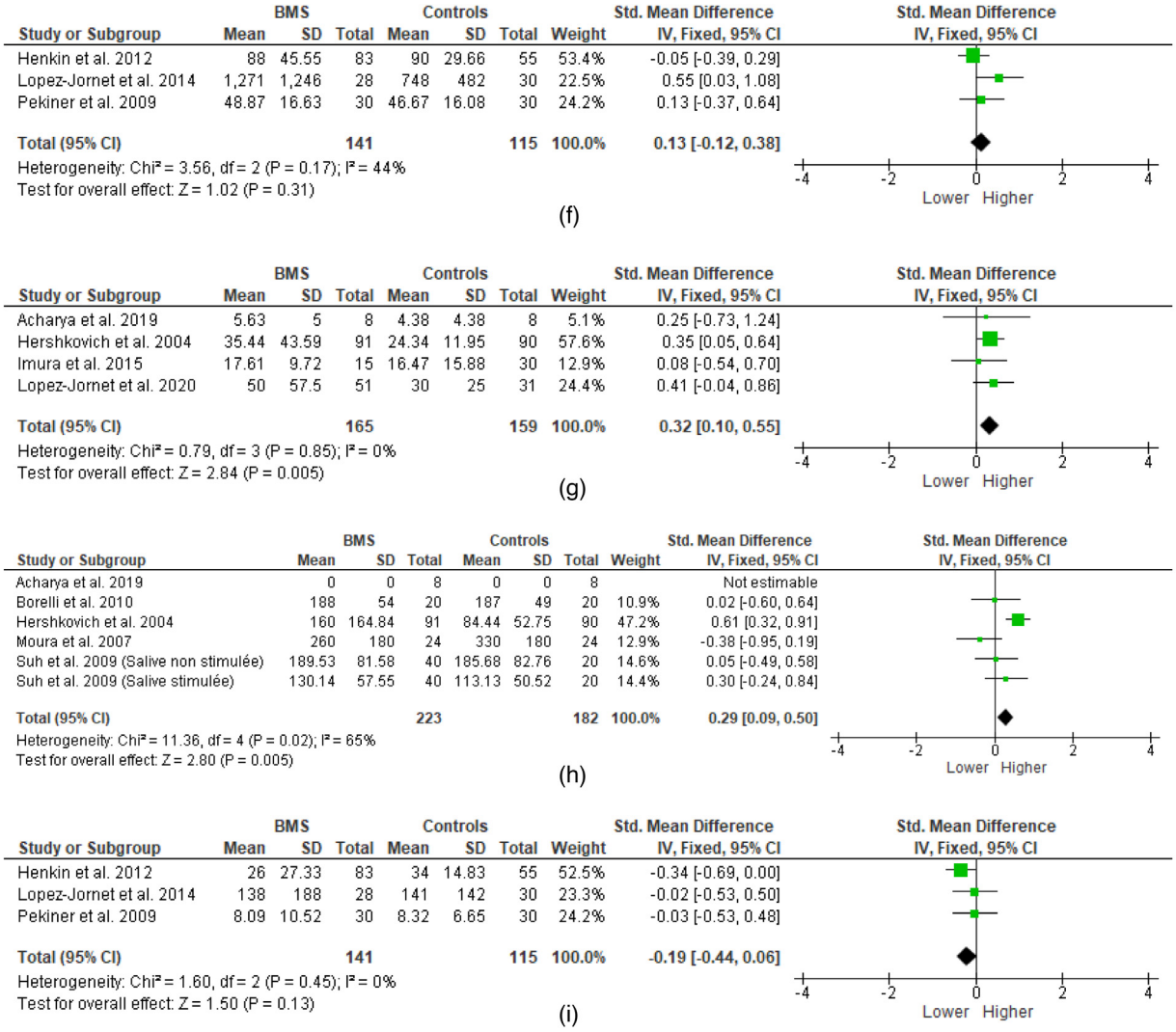



**Fig. 2.** (Continued).

The lack of standardized protocols for the collection of saliva samples is finally what emerges from this work, it therefore appears essential to set up a precise collection technique to obtain significant results.

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## Conclusion

This systematic review confirms the interest of focusing on salivary biomarkers in BMS patients. Nevertheless, the number of available studies being low and of variable methodological quality with a limited number of patients, additional studies are necessary to give a firm and definitive conclusion.

## Conflict of interest

The authors declare that they have no conflicts of interest in relation to this article.

## Funding

The authors declare that no funding was received in regard to this article.

## Ethical approval

We conducted a systematic review and a meta-analysis according to the Preferred Reporting items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) statement. This

type of study does not require an ethical agreement. Nevertheless, the proposed systematic review was registered in PROSPERO under registration number CRD42023403447.

## Informed consent

Not applicable.

## Author's contribution

LD conceived the idea. LD and FK conducted the systematic review. FK and LD led the writing. CGR contributed to the writing and critically revised the manuscript.

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