RESEARCH ARTICLE

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Comparison of Salivary Electrolytes Profile in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

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Abstract

Objectives: to determine salivary electrolyte concentration of oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC) patients. A related systematic review was performed. **Methods:** Observational study. Unstimulated saliva from 18 patients with OSCC, 18 with OPMD, and 18 without oral lesions was collected. A biochemical analysis was performed to evaluate the salivary concentrations of potassium (K), phosphorus (P), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe). Kruskal–Wallis test was performed, and $p < 0.05$ was interpreted as statistically significant. The literature search for the systematic review retrieved 9 studies that associated salivary electrolyte levels with presence and progression of OSCC. **Results:** A highly significant increase was found in the salivary Mg levels in the OPMD group (5.41 µg/mL) in comparison with the OSCC (3.71µg/mL) and control group (3.51 µg/mL) ($p = 0.041$). No differences were observed in other salivary levels elements. The results of the systematic review revealed that one article indicated a decrease, and three papers reported an increase in salivary Na levels in patients with OPMD and OSCC. Two articles indicated a decrease in salivary K levels in OSCC, and the other two reported high Mg levels in OPMD and OSCC. **Conclusion:** High salivary Mg levels can be a potential biomarker indicating the presence of OPMD, however, the evidence is still contradictory and more studies are required.

Keywords: Trace elements- cancer- oral- precancerous condition- electrolytes- saliva

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity. Even with the significant advances in knowledge of this disease, its five-year survival remains approximately 50% due to its aggressive and invasive behavior (Ferlay et al., 2014). Oral potentially malignant disorders (OPMD) are tissue changes that may precede OSCC, and their early detection is essential to prevent their progression to overt OSCC (Van de Waal, 2009; Kujan et al., 2006).

Medical science has focused on finding alternative or complementary methods for diagnosing OSCC in the early stages. In this sense, the use of saliva as a source of biomarkers can be useful due to its advantages, such as ease of evaluation, non-invasive collection, and simple storage (Yoshizawa et al., 2013). Moreover, saliva compounds are characterized by a relatively long shelf life compared to blood (Zhang et al., 2016). The salivary biochemical composition can vary according to the general and oral health of individuals, and its evaluation can be helpful in determining the local and systemic status of patients (Zalewska et al., 2019).

In the case of OSCC, some salivary ions have been associated with the progression to OSCC such as magnesium (Mg) and zinc (Zn). Mg may be involved in tissue changes related to tumorigeneses (Wolf et al., 2007) and Zn regulates the function of metalloproteinases of the extracellular matrix that are involved in tumor progression. Copper (Cu) levels could be related to histodifferentiation in OSCC and zinc (Zn) levels could decrease in patients with OPMD (Ayinampudi and Narsimhan, 2012). Higher levels of sodium (Na) and calcium (Ca) have also been described in OSCC (Dziewulska et al., 2013). However, studies that evaluated several salivary ions simultaneously that may be associated with the progression of OSCC are still very scarce and contradictory. Considering the possible oral biochemical changes that patients with OPMD and

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OSCC can present and those that can be detected in saliva, and their potential use as possible predictors of OSCC (Dziewulska et al., 2013), this study aimed to evaluate the salivary concentrations of potassium (K), phosphorus (P), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) simultaneously in these patients. In addition, obtained data were compared with those obtained in a related systematic literature review.

Materials and Methods

This study comprised two phases. Phase 1 is involved in a systematic review whose main outcome was the use of salivary electrolytes as biomarkers of OSCC progression. Phase 2 is involved in a case-control study whose aim was to characterize the saliva of patients with OPMD, and with OSCC and without oral lesions.

Phase 1: Systematic Review
Protocols and sources of information

This systematic review was conducted according to the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2011), following the four-phase flow diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Moher et al., 2015). The literature search was carried out by two reviewers (J.P.A.S and A.C.U.V) in January 2020. The following databases were screened: PubMed (National Library of Medicine), Scopus (Elsevier), and Web of Science (Thomson Reuters). In addition, the reference lists of the selected articles were searched manually for any missed articles.

Electronic searches

The search strategy is described in Supplementary Appendix 1. The main outcome was case-control studies that considered the use of salivary electrolytes as predictive biomarkers of OPMD progression. The study characteristics were independently extracted by J.P.A.S and A.C.U.V. Any disagreement between the two reviewers was resolved through discussion and consensus. In cases of disagreement, a third reviewer (S.B.C.T) decided whether the article/s should be included. All article titles and abstracts were analyzed and selected in accordance with the eligibility criteria. If the titles/abstracts were unavailable or did not provide sufficient information to decide whether to include or exclude the articles, full-text versions were retrieved. Full-text articles, without language or date restrictions, were then obtained, and the same eligibility criteria as described above were applied. Duplicated articles were excluded.

Data extraction and synthesis

The following information was extracted from each included article: author/s and year of publication, the country where the study was undertaken, gender and mean age of participants, relevant habits (tobacco and alcohol), site of the lesion, and main findings related to salivary electrolyte levels. Due to the high degree of heterogeneity in terms of different studies and methodologies, conducting a meta-analysis was considered inappropriate. The data were descriptively analyzed.

Study types

Studies were excluded for the following reasons: A) studies that used other biological media, such as blood or other body fluids instead of saliva; B) reviews, personal communications, book chapters, and conference summaries; C) *in vitro* studies or *in vivo* animal studies; D) studies with insufficient information on the criteria for the diagnosis of OSCC; E) studies that did not use a histological diagnosis of epithelial dysplasia for OPMD.

Risk of bias assessment

The first two authors systematically assessed the quality of the individual studies by using the JBI Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI). The questionnaire consists of 10 questions are answered with yes, no, unclear, or not applicable. The studies were classified as follows: high methodological quality (>5 "yes" responses), moderate methodological quality (3–4 "yes" responses), or low methodological quality (0–2 "yes" responses) (Moola et al., 2017).

Phase 2: Case-control study
Study design, sampling, and setting

The present study was approved by the Research Ethics Committee of the National Research Ethics Commission (approval code: No. 2.262.681) and conducted following the guidelines of the Declaration of Helsinki. A total of 54 adults, of both sexes, who were referred to the Diagnostic Center for Oral Diseases (DCOD) of the School of Dentistry - Federal University of Pelotas, from 2016 to 2018, were selected. The sample was divided into 3 groups: 1) 18 patients with histological diagnoses of OSCC, 2) 18 patients with oral lesions and a histopathological diagnosis of epithelial dysplasia (and a clinical diagnosis of OPMD); and 3) 18 patients without oral lesions. Before being included, all volunteers were evaluated by an oral pathology specialist. To be included in the experimental group, the patients must have had an indication for biopsy for clinical appearing lesions probably compatible with a histological diagnosis of epithelial dysplasia or OSCC. To be part of the control group, the patients could not have any evidence of oral lesions. The exclusion criteria for all groups were: a history of radiation therapy to the head and neck region; chronic thyroid disease; known Sjogren's disease; those who had prior surgery on the salivary glands; contact allergies; pregnant women; and individuals who currently use antibiotics, corticosteroids, or antifungals.

The final histopathological diagnoses were confirmed by a professional with experience in oral pathology (S.B.C.T). OPMD was histologically classified according to the binary classification system of oral epithelial dysplasia (Kujan et al., 2013). The OSCC staging was performed considering the following three criteria: T (size of the primary tumor), N (spread of the disease to regional lymph nodes), and M (presence of metastasis). Data related to disease and systemic disorders and drug use were obtained from questionnaires. The Eleventh Revision of

the International Classification of Diseases (ICD-11) was used as the criteria for defining alcohol habits (Saunders et al., 2019). Smoking and alcohol habits were recorded. Individuals were classified as smokers if they reported having smoked more than 100 cigarettes in their lifetime and who currently smoke every day (Porter et al., 2003).

Salivary collection

Saliva was collected before the biopsy from patients who gave written informed consent, and salivary electrolyte evaluation was performed only after confirming the histological diagnoses of OSCC, OPMD, and the normal tissues. Previously trained dentists collected unstimulated saliva from patients in a 50 mL pre-weighed Falcon® centrifuge tube. Unstimulated saliva was collected from patients who have not eaten, smoked, or undertaken any oral hygiene 90 min before the procedure (Navazesh et al., 1992). Saliva collection was performed between 9 am and 11 am for 5 min. Subsequently, the tubes with saliva were kept in a container at 5°C for transport to the laboratory of DCOD/UFPel. Each tube was later weighed through gravimetry. A specific weight of 1.005 g/mL was assigned to the fluid, and the calculated total volume was expressed in milliliter per minute to determine the unstimulated saliva flow rate (uSFR). The saliva was centrifuged at 2,500 rpm for 10 min at −5°C, and the supernatants were stored frozen at −80°C until the biochemical analysis. All saliva samples were only used for this investigation. After laboratory analysis, the samples were discarded according to resolution CNS 441 of 2011 (Marodin, Salgueiro, Motta and Santos, 2013).

Salivary biochemical analytical examination

Salivary electrolyte concentration was determined using an inductively coupled plasma optical emission spectrometer (Spectro CIROS CCD, Spectro Analytical Instruments, Kleve, Germany) equipped with a cross-flow nebulizer coupled to a double-pass Scott-type spray chamber. Instrumental performance was optimized following the instructions of the manufacturer and previous work published in the literature (Pereira, Crizel, Novo, Santos and Mesko, 2019). The equipment was externally calibrated using K, P, Na, Ca, Mg, Zn, Cu, and Fe (1.0–10000 μg L^{-1}) reference solutions, which were prepared by diluting a stock solution (1,000 mg L^{-1}, Merck) in 5% HNO_3. The same stock solution was used in the recovery tests for evaluating the accuracy of the determination step. Argon 99.996% (White Martins, São Paulo, Brazil) was used for plasma generation, nebulization, and as an auxiliary gas. For the elemental determination, 100 μL of saliva supernatant was diluted 80 times before the sample introduction into the equipment. This dilution factor was previously optimized to minimize interference during the determination step. The wavelengths selected in the determination step were 589,592 nm for Ca, 766,490 nm for K, 317,933 nm for Ca, 324,752 nm for Cu, 238,204 nm for Fe, 285,213 nm for Mg, 213,857 nm for Zn, and 214,914 nm for P. The results were relatively expressed as μg of the element per mL of saliva. The limit of quantification was calculated from the mean of the curve blank values plus 10 times the standard deviation obtained for 10 replicates of the curve blank.

Data analysis

Descriptive and quantitative data analyses were performed using the Statistical Package for the Social Sciences for Windows 22.0 (SPSS, Inc., Chicago, IL, USA). To determine whether the variables had a normal distribution, the Shapiro-Wilk test was applied. The Kruskal–Wallis test was conducted for comparisons of salivary electrolyte concentrations and uSFR among the groups and between the sex and habits subgroups in each group. A $p < 0.05$ was interpreted as statistically significant.

Results

Phase 1

A total of 881 references were identified in the three electronic databases. Two references were identified through a manual search. After the removal of 51 duplicates, 832 titles/abstracts were evaluated. From those primarily selected, 26 articles agreed the eligibility criteria and were selected for further analysis. After a thorough reading of these articles and the exclusion of those that did not meet the eligibility criteria, 9 full texts were finally included in the final analysis. The flow chart of the study selection is presented in Figure 1.

All articles were published in English between 2002 and 2018. A total of 260 OSCC, 310 OPMD, and 260 control cases were evaluated. All articles evaluated salivary electrolytes in patients with OSCC compared with a control group without oral lesions. Six articles also included patients with OPMD. According to the articles that specified the sex of patients, from 108 OSCC cases, 76 were men (70.4 %) and 32 were women (29.6%); from 35 OPMD cases, 24 were men (68.6%) and 11 were women (31.4%); from the 75 control cases, 47 were men (62.7%) and 28 were women (37.3%). The average age established according to the articles indicating this parameter was 60.3 (35–72) for OSCC, 50.2 (23–64) for OPMD, and 48.5 (37–70) for control patients. Four articles specified the site of the lesions and the site most affected by OSCC was the tongue with 42 (43%) cases. Five articles considered the habits of tobacco and/or alcohol consumption and the percentages of patients with these habits varied between 12.5% and 76.5% for tobacco and between 0% and 13.5% for alcohol among OSCC patients. The only article that referred to habits in patients with OPMD indicated that 100% smoked and nobody (0%) drank.

Eight different salivary electrolyte levels were evaluated. One article indicated a decrease and three an increase in the Na index in patients with OSCC and OPMD (when compared with control group). Two articles presented high salivary Mg levels in patients with OSCC, and one indicated low Mg levels in patients with OSCC and OPMD. One article reported low Zn salivary levels in patients with OPMD and OSCC. Another revealed such levels are higher in patients with OPMD and OSCC than in control group. All information about the characteristics of the included studies is presented in Table 1.

Table 1. Review of Previous Studies Associating the Use of Salivary Electrolytes as Biomarkers or Progression Predictors Associated with OSCC

Study	Method and Patient	Sex | Male: Female	Age Range	Site	Habit		higher levels	lower levels
					Smoke	Alcohol		
Girja KP et al, 2002 - India	1) 15 Control 2) 15 OPMD 3) 15 OSCC	10:05 9:06 12:03	(40−65) (40−64) (45−65)	N/S N/S Buccal Mucosa: 13 (86.7%) Tongue: 2 (13.3%)	0 (0%) 15 (100%) 15 (100%)	0 (0%) 0 (0%) 0 (0%)	N/S	Na and K in OSCC and OPMD
Shpitzer T et al, 2007 - Israel	1) 25 Control 2) 25 OSCC	N/S 12:13	Median 68 ± 17 (30−86) Median (50 ± 15)	N/S Tongue: 25 (100%)	2 (8%) N/S	0 (0%) N/S	Na, Ca, P, and Mg in OSCC	K in OSCC
Fuchs PN et al, 2011 - Croatia	1) 24 OSCC 2) 24 Control	20:04 9:15	(60 ± 2.5) (24 ± 3.7)	Tongue: 4 (16.7%) Sublingual area: 1 (66.6%) Soft palate: 4 (16.7%) N/S	21 (12.5%) 9 (37.5%)	N/S N/S	Na and Cl in OSCC	N/S
Ayinampudi BK et al, 2012 - India	1) 6 Control 2) 20 OPMD 3) 10 OSCC	3:03 15:05 5:05	(38−52) Median (45 ± 5.1) (23−62) Median (45 ± 12.1) (35−65) Median (52 ± 11.5)	N/S N/S N/S	0 (0%) N/S N/S	0 (0%) N/S N/S	Cu and Zn in OPMD and OSCC	N/S
Dziewulska A et al, 2013 - Polonia	1) 30 Control 2) 34 OSCC	25:05:00 27:07:00	(37−70) (35−72)	N/S Tongue: 11 (32.4%) Floor of the mouth: 8 (23.5%) Tonsil: 6 (17.6%) Others: 9 (26.5%)	Smoke or Ex smoke: 17 (56.7%) Smoke or Ex smoke: 26 (76.5%)	N/S N/S	Na in OSCC	-
Shetty SR et al, 2014 - India	1) 65 Control 2) 115 OMPD 3) 50 OSCC	Unspecified sex distribution (20−60) N/S N/S		N/S N/S N/S	N/S N/S N/S	N/S N/S N/S	Zn in OSCC and OPMD	
Shetty SR et al, 2015 - India	1) 50 Control 2) 100 OMPD 3) 50 OSCC	Unspecified sex and age distribution. Matched in age and gender. N/S N/S Unspecified sex distribution:		N/S N/S N/S	N/S N/S N/S	N/S N/S N/S	Cu in OPMD and OSCC	Zn in OPMD and OSCC
Carausu E et al,2016 - Romania	1) 28 Control 2) 43 OPMD 3) 35 OSCC	N/S N/S N/S	51.9 (± 17.1) 52.1 (± 15.5) 55 (± 16.3)	N/S N/S N/S	N/S N/S N/S	N/S N/S N/S	Mg in OSCC and OPMD	
Aziz NZ et al, 2018 - India	1) 17 Control 2) 17 OPMD 3) 17 OSCC	Unspecified sex and age distribution N/S N/S	N/S N/S	N/S N/S N/S	N/S N/S N/S	N/S N/S N/S		Mg in OSCC, < OPMD, < Healthy

OSCC, oral squamous cell carcinoma; OPMD, oral potentially malignant disorders; oral submucous fibrosis; OLP, oral lichen planus; OL, oral leukoplakia; K, potassium; P, phosphorus, Na, sodium; Ca, calcium; Mg, magnesium; Zn, zinc; Cu, copper; Fe: iron; Cl, Chloride; >: higher salivary element levels with statistically significant differences than control group or than group indicated group; <: lower salivary element levels with statistically significant differences than control group or than indicated group. N/S, Not specified

The risk of bias analysis revealed that the main concern regarding the included studies was the identification and handling of the confounding factors among cases and controls to interpret the results. Seven studies were classified as of high methodological quality, and two as of moderate methodological quality. Exposure measures and their form of assessment have presented a low risk of bias. Supplementary Appendix A2 presents the risk of bias of the selected studies by the JBI-MAStARI.

Phase 2

Sociodemographic data

Fifty-four patients participated in this study, 27 men (50%) and 27 women (50%). The participants age varied between 31 and 89 years. The mean age was 56.3 years (±14.6); 42.2 (±16.6) in control group, 58.2 (±13.9) in the OPMD group, and 60.1 (±12.6) in OSCC group. Twenty-four (44.4%) were smokers: four (22.2%) from the control group, 6 (33%) from OPMD group, and 14 (77%) from OSCC group. Twenty-two (40.7%) were social drinkers: 8 (39%) from control group, 7 (39%) from OPMD group, and 7 (39%) from OSCC group. The most frequent site of the lesions in patients with OSCC was the lateral border of the tongue with 9 (50%) cases, followed by the soft palate with 4 (22.2%) cases. The most frequent site of the lesions in patients with OPMD was the buccal mucosa with 9 (50%) cases, followed by the lateral border of the tongue with 5 (27.8%) cases. Regarding the OPMD cases,

Table 2. Baseline Characterization of the Study Population

Group	Gender male: female	Age	Site	Dysplasia classification	Habit		uSFR* (Average, SD) (mL/min)
					Smoke	Alcohol	
Control (n = 18)	6:12	49.2 (± 16.6)	N/A	N/A	4	8	0.645 (± 0.31)
OPMD (n = 18)	7:11	58.2 (± 13.9)	Buccal mucosa: 9 Tongue: 5 Alveolar ridge Mucosa: 2 Palate: 2 Tongue: 9	Low risk: 10 High risk: 8	6	7	0.514 (± 0.12)
OSCC (n = 18)	14:04	60.1 (± 12.6)	Soft palate: 4 Retromolar trigone: 3 Floor of the mouth: 2	N/A	14	7	0.541 (± 0.29)

OSCC, oral squamous cell carcinoma; OPMD, oral potentially malignant disorders; uSFR*, unstimulated salivary flow rate; N/A, Not applicable

16 (88.9%) were clinically leukoplakias and 2 (11.1%) were erythroleukoplakias. Ten (55.6%) were classified as low-risk dysplasia and 8 (44.4%) as high-risk dysplasia. Considering the OSCC cases, according to the TNM classification, 5 (27.7%) patients were classified as T1N0, 8 (44.4%) as T2N0, one (5.6%) as T1N1, one (5.6%) as T2N1, one (5.6%) as T2N2, and 2 (11.2%) as T4N2. The baseline characterization of the study population is summarized in Table 2. The uSFR averages were 0.645 mL/min for the control group, 0.514 mL/min for OPMD and 0.541 mL/min for OSCC, without significant statistical differences ($p > 0.05$).

Salivary electrolytes

A highly significant increase was found in the salivary Mg levels in the OPMD group (5.41 µg/mL) in comparison with the OSCC (3.71µg/mL) and control group (3.51 µg/mL) ($p = 0.041$). Details of the mean, SD, and median of all evaluated salivary electrolyte levels are shown in Table 3 and Figure 2. No differences were observed for the levels of any electrolyte among the three groups or according to their sex or habits ($p > 0.05$).

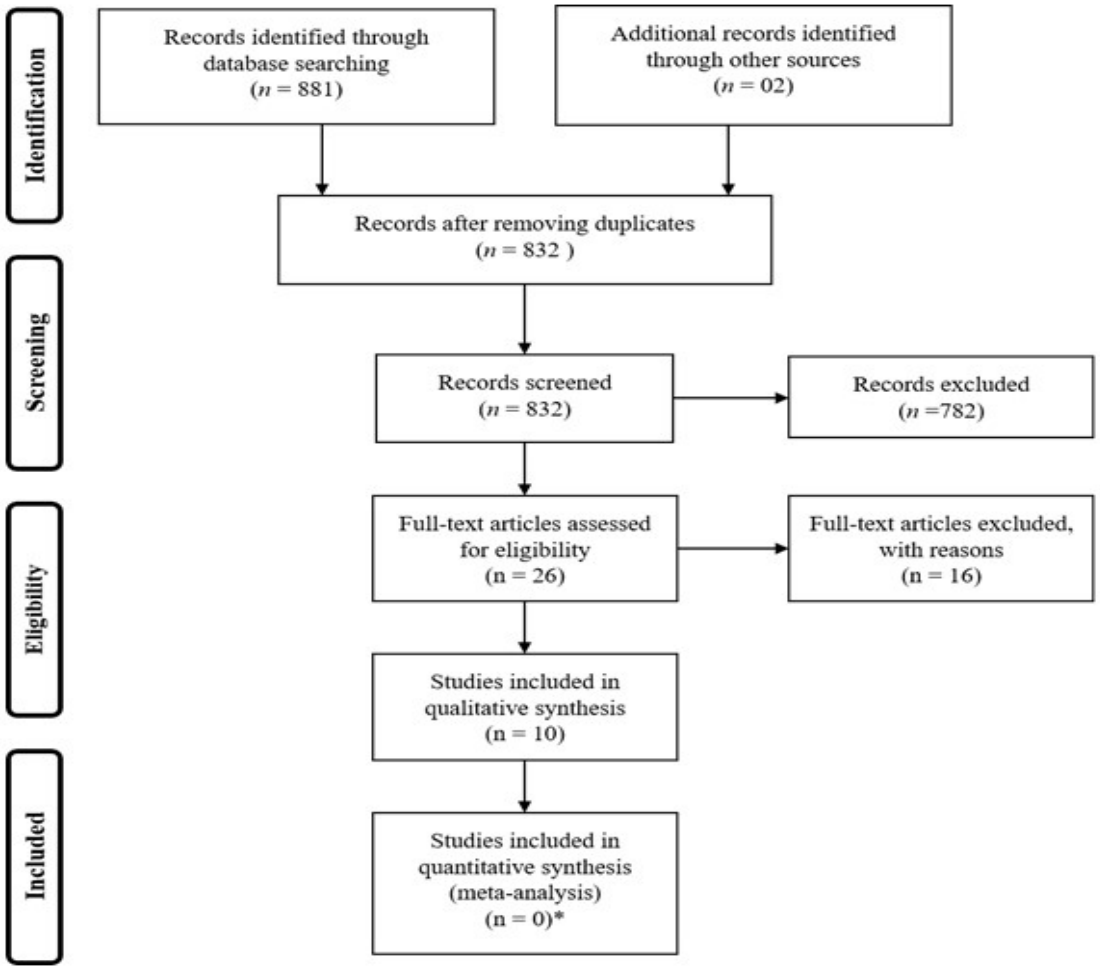

Figure 1. Search Flowchart According to the PRISMA Statement

Table 3. Mean and Standard Deviation of Salivary Electrolyte Levels in Patients without Oral Lesions, with OPMD, and with OSCC

Group	K µg/mL	P µg/mL	Na µg/mL	Ca µg/mL	Mg µg/mL	Zn µg/mL	Cu µg/mL	Fe µg/mL
Control (n =18)	612.6 (± 192.2)	186.7 (± 91.1)	135.4 (± 95.6)	21.8 (± 16.8)	3.51 (± 3.0)	1.15 (± 1.63)	0.61 (± 0.29)	0.27 (± 0.21)
OPMD (n =18)	661.8 (± 158.9)	248.3 (± 82.2)	183.8 (± 202.8)	22.9 (± 14.2)	5.41 (± 4.1)	1.11 (± 1.34)	0.64 (± 0.27)	0.26 (± 0.17)
OSCC (n =18)	651.3 (± 213.4)	237.2 (±109.6)	171.9 (± 92.6)	17.5 (± 11.5)	3.71 (±2.3)	0.87 (± 0.82)	0.51 (± 0.82)	0.26 (± 0.15)
P-value	0.459	0.136	0.469	0.359	0.041*	0.917	0.132	0.908

K, Potassium; P, Phosphorus; Na, Sodium; Ca, Calcium; Mg, Magnesium; ZN, Zinc; Cu, Copper; Fe, Iron; OPMD, Oral potentially malignant disorders; OSCC, Oral squamous cell carcinoma; *, Statistically significant

Discussion

Several metabolic disorders, oral precancerous conditions, and oral cancers are accompanied by alterations in the concentration of one or more salivary trace elements. Their identification is helpful in establishing an early diagnosis, which leads to early initiation of treatment, and also in the prognostication and in tracking the disease progression (Hosthor et al., 2014).

K, Na, Ca, Cl, and P are the most concentrated biochemical elements in saliva due to their importance in the electrolytic balance of this fluid. According to sociodemographic and habits characteristics of the sample, the present study agrees with previous studies that there would be no differences according to sex (Rutherfurd-Markwick et al., 2017) or smoking and alcohol habits (Avşar et al., 2009). The differences in electrolytes salivary levels could be determined by other factors, such as oral health. Mg was the only electrolyte that showed different salivary levels among the studied groups. Its salivary concentration in the OPMD group was higher than in the other groups. Mg is a mineral required for a wide variety of physiological functions and biological activities, such as activation or inhibition of enzymes and regulation of cellular proliferation, progression, and differentiation (Al Alawiet al., 2018).

Currently, attention is being directed to the role of Mg in tumor biology because of its involvement in processes such as proliferation, cell death, de-differentiation, invasion, and neoangiogenesis (Wolf et al., 2007). In the evaluation of Mg concentration and its relationship with different types of cancer, factors such as the presence of this electrolyte in foods in the daily diet should be considered. In this regard, studies have generally demonstrated beneficial effects of Mg intake and cancer, such as an inverse relationship between high Mg levels in drinking water and less chance of dying because of

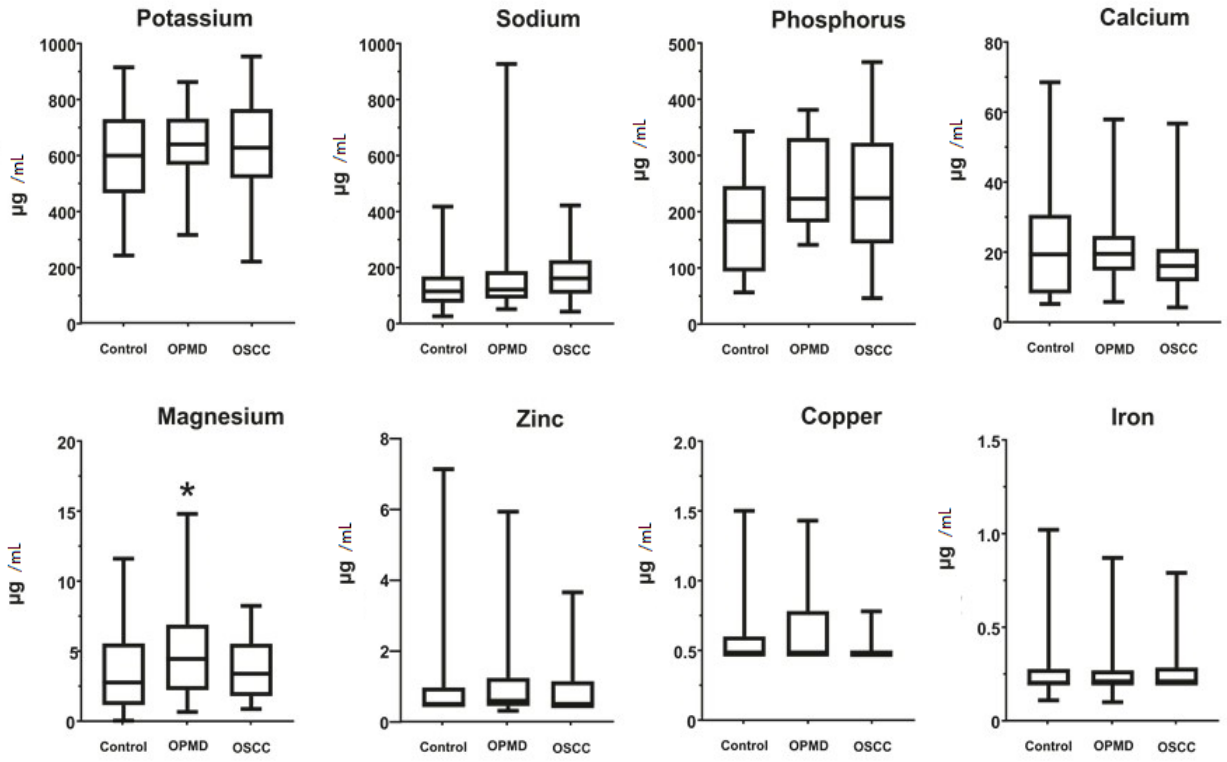

Figure 2. OPMD, Oral potentially malignant disorders; OSCC, Oral squamous cell carcinoma; *, Statistically significant

breast cancer (Yang et al., 2000) or ovarian cancer (Chiu et al., 2004). However, other evidence indicates that no association exists between the risk of developing bladder cancer (Michaud et al, 2000) or gastric cancer (Pasternak and Prztszlak, 1999) and the total dietary intake of Mg.

The reports of salivary and serum Mg levels and their relationship with OSCC development, although equivalent in these two fluids, is contradictory. Some researchers (Shpitzer et al., 2007; Carausuet al., 2016) found that salivary Mg levels were higher in patients with OSCC or OPMD than in control group. In contrast, other researchers (Aziz et al., 2018) showed that Mg levels are low in the plasma and saliva of OSCC patients. A concluded that the salivary Mg level could be a risk factor for the progression of tumorigenic activities in head and neck carcinoma (Nasulewicz et al., 2004). Once established, OSCC may influence the availability of plasma Mg, enhancing angiogenesis but, in a contradictory effect, also inhibiting endothelial cell migration and proliferation (Banai et al.,1990). Mg can improve angiogenesis by increasing nitric oxide and vascular endothelial growth factor production (Maier et al., 2004). In animals subjected to a low Mg diet, a return to a normal diet induces an almost explosive tumor growth that can be explained, at least in part, by a potentiation of angiogenesis.

After 40 years of age, there is an increase in cortical glomerulosclerosis and a decline in both glomerular filtration rate and renal plasma flow (Schlanger et al., 2010). These changes may be associated with an inability to excrete specially ammonium, Na, or K average age of control group and OPMD group in this study, although lower than that of patients with OSCC, so far exceeds the average of 40 years. Thus, this factor would not be decisive to explain electrolytes levels differences established between groups.

Salivary levels of the other trace elements evaluated in this study showed no significant differences among the evaluated groups. In the population over 65 years of age, there are physiological changes among which are alterations in the levels of different electrolytes and that are related to altered perception of thirst, decreased glomerular filtration rate and limitations in the excretion of water, Na, K, among other elements (Luckey et al., 2003). It is then possible that the loss of balance and homeostasis of the electrolytes present in the body only become evident at ages above the average of the patients evaluated in the present study, except in the case of Mg, already discussed. The need for maintenance of electrolytes in balanced levels due to the importance that these have in different metabolic processes, could explains the lack of difference that they evidenced when comparing the different groups in this study. The loss of this balance could resulting increase in morbidity and mortality, especially in elderly people (Allison et al., 2004). A study included in the systematic review reported a similar result, indicating that the assessment of Na, K, Ca, Fe, P and even Mg salivary levels as biomarkers are not useful in OSCC (Dziewulska et al., 2013). However, some studies showed differences between the salivary electrolytes evaluated, as well as a higher concentration of Cu in patients with OSCC (Khanna, 2008). In the case of Zn, one study showed a higher (Shetty et al, 2014) and another, lower (Shetty et al., 2015) salivary concentrations of this electrolyte in patients with OSCC and OPMD than control group. Zn is essential for regulation of the cell cycle and cell division, for DNA polymerase activity, and is particularly important for the rapid cell proliferation encountered in growing tumors (Ayinampudi and Narsimhan, 2012). However, differences in the salivary concentrations of this electrolyte were not observe. According to the systematic review, high concentrations of Na salivary levels in patients with OSCC are reported in some studies (Shpitzer et al., 2007; Fuchs et al., 2011), a finding that could reflect dehydration due to smoking and alcohol consumption; however, a different salivary levels of Na between groups was not observed.

Although an equivalence between the levels of electrolytes in the blood and saliva was indicated in the systematic review suggesting the use of saliva as a source as biomarkers (Aziz et al., 2016), it was not possible to conduct this analysis in our case-control study. That was one of the limitations of this study. Another limitation could be the sample size, which ideally should be larger, and the differences between the means of age between the study groups, a phenomenon that was explained in detail. Finally, it should be mentioned that not having considered the type and quality of diet of the patients evaluated, may be a factor that also affects the results obtained, so it should be an important element to consider in future studies. However, the higher levels of Mg found in the saliva of patients with OPMD may be of great interest. One possible hypothesis for this observation is that the increase in this electrolyte could be an initial angiogenic stimulus in the malignancy process from OPMD, but later, factors other than Mg levels could be involved in the maintenance of neovascularization in OSCC. Further study is necessary to confirm this theory and to better understand the role of this electrolyte in the progression to OSCC. In this sense, it would be highly recommended to associate Mg levels in different body fluids with the density of blood vessels in the different groups of patients studied. More research complemented with in vivo studies must be performed to correlate the local neoplastic behavior of OSCC with different concentrations of salivary electrolytes. Even though saliva represents a source of biomarkers that may reflect the progression of OSCC, and elements such as Mg may be of great interest in this regard, additional studies must be conducted.

Author Contribution Statement

All authors approved the final version of the manuscript. Conceptualization: [Juan Aitken-Saavedra]; Methodology: [Diogo La Rosa Novo, Marcia Foster Mesko]; Formal analysis and investigation: [Ricardo Fernandes Ramires, Sandra Beatriz Chaves Tarquinio]; Writing - original draft preparation: [Juan Aitken-Saavedra]; Writing - review and editing: [Juan Aitken-Saavedra, Diogo La Rosa Novo, Marcia Foster Mesko, Ana Carolina Uchoa Vasconcellos, Karine Duarte da Silva, Gabriel Rojas Zuñiga, Ricardo Fernandes Ramires, Sandra Beatriz Chaves Tarquinio] Supervision: [Sandra Beatriz Chaves Tarquinio].

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Any conflict of interest

None.

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