Salivary cytokines as biomarkers of oral cancer: a systematic review

Mayara Martina Abatti Chiamulera  
Universidade do Oeste de Santa Catarina

Caroline Biazzolo Zancan  
Universidade do Oeste de Santa Catarina

Aline Pertile Remor  
Universidade do Oeste de Santa Catarina

Frederico Omar Gleber-Netto  
University of Texas MD Anderson Cancer Center

Antuani Rafael Baptistella  
AC Camargo Cancer Center  
antuani.baptistella@unoesc.edu.br

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Abstract

**Background:** Oral cancer (OC) is usually diagnosed at advanced clinical stages due to its asymptomatic nature and absence of pathognomonic signs in its early development phase. Delayed diagnosis is one of the major causes of OC treatment failure and poor prognosis. Development of alternative diagnostic approaches are imperative for improving early detection and therapeutic success rates. Salivary cytokines (SC) have been studied as potential diagnostic biomarkers for OC and may represent a potential tool for improvement of OC early detection.

**Methods:** In this systematic review we identified SC studied as OC biomarker by systematically reviewing the PubMed and Cochrane Library databases using the terms: "oral cancer", "cytokine", and "saliva", and also combined with "interleukin" or "interferon". Only case-control studies that measured SC by ELISA from treatment naïve patients were included.

**Results:** A total of 28 articles (from 2004 to 2018) were included, describing 10 different SC, which IL-8 and IL-6 were the most studied ones. SC levels were consistently higher among OC patients when compared to healthy controls and to patients with non-malignant oral conditions such as leukoplakia, lichen planus and gingivitis, irrespective of its inflammatory role.

**Conclusions:** Our analysis suggests SC are consistently up-regulated among OC patients and may represent ideal biomarkers to be translated to the clinical practice.

1. Background

Oral squamous cell carcinoma (OSCC) is responsible for approximately 2% of all cancer cases in the USA, and it is usually diagnosed at advanced clinical stage[1]. Advanced disease is the most important factor of increased morbimortality among OSCC patients. Treatment of large and disseminated tumors requires wider surgical resections, ionizing radiation exposure and systemic administration of cytotoxic drugs, leading to major cosmetic, functional and psychological damage to these patients. Despite of being aggressively treated, patients with advanced OSCC still have a worse prognosis than patients with small and localized tumors [2]. The five-year survival rate for advanced OSCC patients is around 50%, while about 85% of patients diagnosed at early stages are still alive by the fifth year of follow-up (3,4). Thus, early diagnosis and treatment are the major determinants for cancer cure, improved quality of life and survival.

Unfortunately, early OSCC diagnosis represents a major clinical challenge. Early stage lesions are usually asymptomatic and are rarely perceived by patients. Thus, patients rarely seek medical attention reporting oral lesions, and diagnosis of early stage oral cancer is usually incidental. Additionally, the clinical appearance of initial oral cancer lesions may be misleading even for trained professionals. Early OSCC lesions may be clinically indistinguishable from other benign oral mucosal conditions, histopathological assessment is imperative for definitive diagnosis [4, 5].

Many of OSCC cases are preceded by oral mucosa disorders with considerable potential for malignant transformation, which are collective named as potentially malignant oral disorders (PMOD). Despite its risk of malignant development, only 2% of PMOD will eventually turn in to cancer. Prediction of cancer risk for PMOD patients, as well as, differentiation between PMOD and early cancer lesions, are solely based on clinician experience and histological predictions, which are poorly accurate [6–8].

Considering that all these factors contribute to further delay cancer diagnosis, the field has been trying to identify biomarkers that could assist in the early detection of OSCC, and differentiate it from benign lesions with similar clinical features. Because saliva is in intimate contact with the oral mucosa, it has been widely studied as source of oral cancer biomarkers [9]. Saliva carries molecules and cells originated in the aero-digestive tract, but also, nucleic acids and proteins that are passively and actively transported from the circulatory system into the salivary glands structures [10]. In this way, saliva is capable of providing information about the health state of an organism, as blood does, but with the advantage of being obtained by a non-invasive, inexpensive, and safe technique [11].

Early exploratory studies found that salivary cytokines were highly deregulated in oral cancer patients, suggesting they were potential cancer biomarkers. However, despite of having a major role on human carcinogenesis, inflammation is an ubiquitous phenomenon in many human diseases [12]. Subsequent studies attempted to test whether salivary cytokines represented accurate cancer biomarker not only in the context of healthy individuals, but also when compared to patients with inflammatory and benign oral diseases. Despite of that, there is still no consensus on which salivary cytokines shows potential value as a cancer biomarker even in the presence of inflammatory oral diseases. Therefore, the objective of this systematic review is to identify salivary cytokines with potential to be used as oral cancer diagnostic biomarker and verify their performance among different oral conditions. The results presented here are of great
relevance for guiding future technical and clinical endeavor seeking the development of early stage oral cancer biomarker based on salivary cytokines, which is essential to initiate the treatment as early as possible, what can impact in the morbimortality of these patients.

2. Methods

This review was performed following the Cochrane Handbook for Systematic Reviews of Interventions (Version 5.1.0) [13], also registered and published at the International prospective register of systematic reviews (PROSPERO - nº CRD42018111397). Data search, screening and extraction were executed by two of the authors (MMAC and CBZ).

Data search was performed using the electronic databases PubMed and Cochrane Library, considering manuscripts published between 1950 and 2019 and 1999 and 2019, respectively. The searching terms used were: “cytokine”, “oral cancer” and “saliva”, and also combined with “interleukin” or “interferon”. The searching period was from September, 2018 to January, 2019.

The resulting manuscripts were initially screened by title and abstract, followed by a full-text analysis. Manuscripts were then selected based on the following inclusion criteria:

- Publication type: Peer-reviewed original articles, published in the English language;
- Study design: Case-control studies with human subjects
- Exposure of interest: Participants enrolled in the “case” group should have been diagnosed with oral squamous cell carcinoma, while “controls” should be non-cancer patients. For both groups, salivary cytokines should have been measured in its protein form.
- Method of sampling: Salivary cytokines should have been measured using enzyme-linked immunosorbent assay (ELISA).
- Research question: The selected studies should have compared salivary cytokine levels between oral cancer patients and non-cancer control subjects.

In case of discordance about the eligibilities criteria, a third author (ARB) was consulted.

Data extraction

A database was created to organize the information from the selected publications, including: author details, year of publication, number of patients, cytokine expression values, statistical methods and statistical results. Two authors (MMAC and CBZ) analyzed the data and a third author (ARB) re-evaluated all the information.

The selected articles were further evaluated considering the following aspects: the period of sample collection was reported; the study was prospective; saliva collection, storage, and analysis techniques were standardized and properly reported.

Data analysis

Among the included articles, Polz-Dacewicz et al. (2016) reported cytokine concentrations in nanograms of protein per milliliter of saliva (ng/ml), which were converted to picograms of protein per milliliter of saliva (pg/ml) aiming to standardize their findings with the other studies. On the other hand, Gonçalves et al. (2015) reported their results in picograms of cytokine per milligram of total salivary protein (pg/mg) and were not standardized with the other studies.

Aiming to visualize the salivary cytokine expression levels among oral cancer (OC) patients and non-cancer controls (NCC), mean salivary cytokine expression levels of NCC groups were divided by mean expression levels in OC groups for each individual study. NCC groups were categorized according to the following groups: healthy controls; oral lichen planus (OLP) controls, gingivitis controls and potentially malignant oral lesions (PMOL) controls.

3. Results

The initial search in electronic databases PubMed (1950 – 2019) identified 182 studies. After screening, a total of 28 studies were included into our analysis (Figure 1 – Flow chart).

The selected 28 studies: were all published between 2004 and 2018; from 12 different countries; the sample size ranged from 18 to 300 patients. The control groups were composed by healthy individuals (Control), patients with potentially malignant oral lesions (PMOL),
oral lichen planus (OLP), or periodontitis (Table 1). The total number of patients, combining the 28 studies, considering each cytokine evaluated, each study group, and cytokine per study group are described in the Figure 2. IL-8 was investigated in 1245 individuals, followed by IL-6 and TNF-α, investigated in 963 and 724 individuals, respectively (Figure 2A). Between the studied groups, the OC had the higher number of individuals included (1670), while 1574 healthy controls, 667 individuals with PMOD, 108 with OLP and 62 with periodontitis were included in the 28 selected articles (Figure 2B).

The cytokines identified in the frequency of appearance in these studies were: IL-8 (50%), IL-6 (50%), TNF-α (28.6%), IL-1β (21.4%), IL-10 (17.9%), IL-1α (10.7%) and IL-1, IL-1RA, IL-4 and IL-13 (3.6% each) (Figure 2). The mean cytokine expression values for each control and oral cancer groups (Table 1) were used for calculation of the expression ratio between control and cancer groups for each individual study (Figure 3). Since the oral cancer groups were used as denominators, cytokine expression ratio values lower than “1”, were indicative of higher cytokine levels among cancer samples, while ratios higher than “1” indicated higher levels among controls.

Expression levels of IL-8, IL-6, IL-1β, IL-10, IL-1RA, IL-4 and IL-13 were always higher among cancer groups in all studies evaluated (Figure 3). For TNF-α and IL-1α, salivary expression levels were usually higher among OC patients when compared to matching controls. However, Rhodus et al. 2005b (12.5% of all TNF-α studies) and Lee et al. (2018) (33.3% of all IL1α studies) observed higher TNF-α and IL1α expression in healthy controls than in OC patients, respectively (Figure 4C and 4F).

**Interleukin-8**

Salivary interleukin-8 (IL-8) levels in oral cancer patients were compared to controls in 14 articles (Figure 4A). The study with the smaller population of oral cancer included 05 patients (Cheng, S. et al. 2014), and the biggest one, 100 oral cancer patients (Rajkumar, K. et al. 2014). Among these 14 articles, 14 groups of oral cancer, 5 groups of potentially malignant oral disorder (PMOD) patients, 2 groups of oral lichen planus (OLP) and 2 groups of periodontitis patients were evaluated. In all these studies, IL-8 levels were significantly different between OSCC patients and controls. The values of IL-8 in control group varied from 52.1 to 1580.7 pg/ml; for the premalignant group, values between 140.3 and 1918.2 pg/ml were found; and in the oral cancer group, the values were between 283.7 and 4082.8 pg/ml [14, 15, 24–27, 16–23].

**Interleukin-6**

Interleukin-6 (IL-6) was evaluated in 14 articles, and in all of them the amount of IL-6 was statistically higher in oral cancer patients compared to the control group (Figure 4B). The population of the studies vary from 9 subjects in the smallest study, to 100 subjects in the biggest one. Five articles only compared oral cancer patients with a control group of non-cancer people, while 07 articles also compared the oral cancer with premalignant lesions, and 01 article compared the oral cancer patients with a non-cancer group with periodontitis. The IL-6 values for the control group vary from 0 to 16.0 pg/ml in eleven of the articles, while in two studies it was found in higher values (33.4 and 69.23 pg/ml). For the pre-malignant group, values between 0.431 and 217.8 pg/ml were found in another five studies. In the oral cancer groups, the values were from 0.707 to 435.04 pg/ml, and the values were higher than 80.0 pg/ml in eight studies [14, 18, 32–35, 20, 23, 25, 26, 28–31].

**Tumor necrosis factor (TNF-α)**

TNF-α was analyzed in 08 articles and it was found in significant higher amount in patients with oral cancer, compared to controls, in 7 of these studies (Figure 4C). The study population vary from 09 to 100 subjects. Three studies only compared oral cancer patients with a healthy control group, while 05 articles compared the oral cancer patients with subjects with premalignant lesions. The TNF-α values for the control groups were from 0.013 to 11300 pg/ml. For oral cancer patients, the TNF-α values vary from 0.739 to 23100 pg/ml [23, 26, 31, 32, 36–39].

**Interleukin-1β**

The IL-1β was analyzed in 06 articles and a significant difference between oral cancer patients and control subjects was found in all studies (Figure 4D). The number of subjects included in the studies varied from 28 to 60. Two studies compared oral cancer patients with pre-malignant lesions, 04 studies compared oral cancer patients with healthy subjects. The IL-1β values in the control group varied from 14.1 to 354 pg/ml. In the premalignant group the variation was between 39.6 to 143 pg/ml, while in the oral cancer patients the amount varied from 101.03 to 906 pg/ml [15, 16, 18, 32, 38, 40].

**Interleukin-10**
Interleukin-10 (IL-10) was evaluated in 05 articles and in three of them was observed statistical difference in cancer patients compared to control subjects (Figure 4E). The studies evaluated from 22 to 78 subjects per group. All articles compared oral cancer patients to control subjects. The salivary IL-10 values were between 0.027 and 10.01 pg/ml in control groups, and between 0.037 and 14.91 pg/ml in oral cancer patients [32, 37, 41–43].

Interleukin-1α (IL-1α)

IL-1α was evaluated in 03 articles, two compared oral cancer patients with healthy controls and one study compared with oral lichen planus (Figure 3). Two of these articles presented results with significant difference between oral cancer and controls. The IL-1α quantification varied from 135.88 to 1054.6 pg/ml in control group, was 293.6 pg/ml in oral lichen planus, and between 201.7 and 995.71 pg/ml in oral cancer patients [14, 23, 32].

Other Cytokines

The IL-1RA was found in one study that compared oral cancer patients with healthy controls (Figure 3). The salivary IL-1RA in the oral cancer group was 2831.6 pg/ml, and 1949.2 pg/ml in control group, not showing significant difference between them [43].

The IL-1 (without specification of the subunit) was studied in one article, comparing oral cancer patients with patients with premalignant lesions and a group of healthy controls (Figure 3). It was found differences in salivary IL-1 between groups. The IL-1 value found in oral cancer patients was 454.4 pg/ml, in premalignant lesions was 255.1 pg/ml, and in healthy controls 173.2 pg/ml [26].

The IL-4 was studied in 01 article comparing oral cancer patients with healthy subjects (Figure 3). Oral cancer patients present 1.17 pg/ml of IL-4 in saliva, while healthy subjects 1.01 pg/ml, without statistical difference between groups [43].

The IL-13 also was analyzed in 01 study, comparing the same groups as the previous one (Figure 3). In oral cancer patients the IL-13 level was 0.76 pg/ml, and in the control groups 0.23 pg/ml, showing a difference statistically significant [43].

4. Discussion

Actually, the salivary cytokines are not used in clinical practice to diagnose the oral cancer, however, as observed in the data found in this review, the levels of salivary cytokines present a great potential to serve as a diagnose biomarker of these tumors. There are still technical and biological issues that must be overcome for a final and definitive evaluation of the value of these biomarkers. Despite all the studies analyzed in this review used the ELISA technique, one of the main limitation of the results is the variability in the cytokines values obtained. A metanalysis of 26 studies that all analyzed the cytokine levels in saliva and blood by ELISA, presented this technique as the most accurate to measure proteins in biofluids [49].

Although the relative expression of the salivary cytokines demonstrated a good potential to separate oral cancer patients and non-cancer subjects, and the increased levels of these cytokines in cancer patients are consistent, the use of the salivary biomarkers is not intended to replace the gold standard diagnose of oral cancer, based in clinical examination and biopsy [50]. The use of these biomarkers must be improved to be used specifically in at-risk populations as an auxiliary method for screening and early diagnose.

Although the majority of studies were composed by patients with the disease in advanced stages, some studies showed that the cytokines levels increase as the disease stage advances [22]. Most studies do not separate patients according to disease stage, which limits the homogeneity and standardization of cytokine values, besides these tumors normally are diagnosed in advanced stages [51], cannot be said that is possible identified, through salivary cytokines, initial lesions or early stages tumors.

Another limitation is the ELISA kits used to measure the cytokines in these studies, which are reagents label as “Research Use Only”, indicating that there is no strict regulation on the technical characteristics of the tests. Thus, the comparison of results between different studies is limited, since the variation between brands or even lots can lead to variations in results. For the continuity of the validation process of these salivary cytokines as biomarkers of oral cancer it is necessary the development of studies that consider the variability of the disease presentation, the measurement of the cytokines in an controlled environment, and using reagents developed to clinical use. Moreover, so that possible biases are excluded, multcenter studies must be performed, using a larger sample than the previous studies [52, 53]. And for the results to be reliable, it is of fundamental importance that an international standardization be validated for both saliva collection and salivary cytokines measurements.
5. Conclusion

In this review, we found 28 articles that evaluated 10 different pro and anti-inflammatory salivary cytokines in oral cancer patients. IL-8 and IL-6 were the most studied ones, and in all articles these salivary cytokines were found at higher levels in oral cancer patients compared to non-cancer control subjects. Excepting IL-4 and IL-1RA, all other studied cytokines can be explored in the future to determine the real potential as a biomarker for oral cancer. Furthermore, it was found a big variability in the cytokines values in the different studies, even using the same quantification methodology.

In order to translate these biomarkers into the clinical practice, standardization of saliva collection and cytokines measurement process is required, as well larger and multicenter studies.

Abbreviations

OC = Oral cancer
SC = Salivary cytokines
OSCC = Oral squamous cell carcinoma
PMOD = potentially malignant oral disorders
ELISA = enzyme-linked immunosorbent assay
NCC = non-cancer controls
OLP = oral lichen planus
IL-8 = interleukin-8
IL-6 = Interleukin-6
TNF-α = Tumor necrosis factor α
IL-1b = Interleukin-1b
IL-10 = Interleukin-10
IL-1a = Interleukin-1a
IL-1RA = Interleukin-1RA
IL-4 = Interleukin-4
IL-13 = Interleukin-13

Declarations

Ethics approval and consent to participate: not applicable.

Competing Interests: The authors declare that they have no conflict of interest.

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Availability of data and material: All data generated or analyzed during this study are available for consultation and can be requested from authors.

Authors' contributions: All Authors read and approved the manuscript. MMAC: Study concept and design; analysis and interpretation of data; drafting of the manuscript. CBZ: Study concept and design; analysis and interpretation of data; drafting of the manuscript. APR:
Drafting of the manuscript; critical revision of the manuscript. FOGN: Study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript. ARB: Study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7–34. doi:10.3322/caac.21551.
2. Amit M, Yen TC, Liao CT, Binnenbaum Y, Chaturvedi P, Agarwal JP, et al. Clinical nodal stage is a significant predictor of outcome in patients with oral cavity squamous cell carcinoma and pathologically negative neck metastases: results of the international consortium for outcome research. Ann Surg Oncol. 2013;20:3575–81. doi:10.1245/s10434-013-3044-0.
3. Curado MP, Johnson NW, Kerr AR, Pereira L, Mendonca DR, Lanfranchi H. Oral and oropharynx cancer in South America: Incidence, mortality trends and Oral and oropharynx cancer in South America: Incidence, mortality trends and gaps in public databases as presented to the Global Oral Cancer Forum. Transl Res Oral Oncol. 2016;1:1–7.
4. Bagan J, Sarrion G, Jimenez Y. Oral cancer: Clinical features. Oral Oncol. 2010;46:414–7. doi:10.1016/j.oraloncology.2010.03.009.
5. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin. 52:195–215. doi:10.3322/canjclin.52.4.195.
6. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37:1–10. doi:10.1111/j.1600-0714.2007.00579.x.
7. Warkakulasuriya S. Causes of oral cancer – an appraisal of controversies. Br Dent J. 2009;207:471–5.
8. Schaefer CA, Schaefer JJ, Yakob M, Lima P, Camargo P, Wong DTW. Saliva Diagnostics: Utilizing Oral Fluids to Determine Health Status. 2014. p. 88–98.
9. Katakura A, Kamiyama I, Takano N, Shibahara T, Muramatsu T, Ishihara K, et al. Comparison of Salivary Cytokine Levels in Oral Cancer Patients and Healthy Subjects. Bull Tokyo Dent Coll. 2007;48:199–203.
10. Rajkumar K, Nandhini G, Ramya R, Rajashree P, Sc M, Kumar AR, et al. Validation of diagnostic utility of salivary interleukin-8 in differentiation of potentially malignant oral lesion and malignant oral squamous cell carcinoma in high endemic setting region. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014.
11. Lisa Cheng Y-S, Jordan L, Gorugantula LM, Schneiderman E, Chen H-S, Rees T. Salivary Interleukin-6 and -8 in Patients With Oral Cancer and Patients With Chronic Oral Inflammatory Diseases. J Periodontol. 2014;85:956–65.
12. Punyani SR, Sathawane RS. Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma. Clin Oral Investig. 2013;17:517–24.
22. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Evaluation KWC. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2017.

23. Rhodus NL, Cheng B, Myers S, Miller L, Ho V, Ondrey F. The feasibility of monitoring NF-κB associated cytokines: TNF-α, IL-1β, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus. Mol Carcinog. 2005;44:77–82.

24. Maia A. R. St. John, MD, PhD; Yang Li, DDS, PhD; Xiaofeng Zhou, PhD; Paul Denny, PhD; Chih-Ming Ho, PhD; Carlo Montemagno, PhD; Wenyuan Shi, PhD; Fengxia Qi, PhD; Benjamin Wu, DDS, PhD; Uttam Sinha, MD; Richard Jordan, DDS, PhD; Lawrence Wolinsky, DDS, Ph Dms. Interleukin 6 and Interleukin 8 as Potential Biomarkers for Oral Cavity and Oropharyngeal Squamous Cell Carcinoma. Arch Otológryngol Head Neck Surg. 2004;130:929–35.

25. Khyani IAM, Qureshi MA, Mirza T, Farooq MU. Detection of interleukins-6 and 8 in saliva as potential biomarkers of oral pre-malignant lesion and oral carcinoma: A breakthrough in salivary diagnostics in Pakistan. Pak J Pharm Sci. 2017;30:817–23.

26. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-κB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev. 2005;29:42–5.

27. Gleber-Netto FO, Yakob M, Li F, Feng Z, Dai J, Kao HK, et al. Salivary biomarkers for detection of oral squamous cell carcinoma in a Taiwanese population. Clin Cancer Res. 2016;22:3340–7.

28. Sato J, Goto J, Murata T, Kitamori S. Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;110:330–6.

29. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17.

30. Selvam NP, Sadaksharam J. Salivary interleukin-6 in the detection of oral cancer and precancer. Asia-Pacific J Clin Oncol 2015;2015;11:236–41.

31. JURETIć M., R. CEROVIć, M. BELUŠIć-GOBIć, I. BREKALO PRŠO2, L. KQIKU3, S. ŠPALJ4. Salivary Levels of TNF-α and IL-6 in Patients with Oral Premalignant and Malignant Lesions. Folia Biol 59,. 2013;59:99–102.

32. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2018;47:699–707.

33. Zhang S, Zhang X, Yin K, Li T, Bao Y, Chen Z. Variation and significance of secretory immunoglobulin-A, interleukin 6 and dendritic cells in oral cancer. Oncol Lett. 2017;13:2297–303.

34. Dineshkumar T, Ashwini BK, Ramesh Kumar A, Rajashree P, Ramya R, Rajkumar K. Salivary and Serum Interleukin-6 Levels in Oral Premalignant Disorders and Squamous Cell Carcinoma: Diagnostic Value and Clinicopathologic Correlations. Asian Pac J Cancer Prev. 2016;17:4899–906.

35. Bagán L, Sáez GT, Tormos MC, Labaig-rueda C, Murillo-cortes J, Bagan J V. Salivary and serum interleukin-6 levels in proliferative verrucous leukoplakia. Clin Oral Invest DOI. 2015.

36. Sahebjamee M, Eslami M, Atarbashimoghadam F, Sarafnejad A. Salivary concentration of TNF-α, IL1β, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008;13:292–5.

37. Polz-dacewicz M, Strycharz-dudziak M, Dworza J, Stec A. VEGF levels in oropharyngeal squamous cell carcinoma and correlation with HPV and EBV infections. Infect Agent Cancer. 2016;11:1–8.

38. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17:e10-5.

39. Krishnan R, Thayalan DK, Padmanaban R, Ramadas R, Anasamy RK, Anandan N. Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating premalignant and malignant oral disease. Asian Pacific J Cancer Prev. 2014;15:7141–8.

40. Gleber-netto FO, Yakob M, Li F, Feng Z, Dai J, Kao H, et al. Salivary biomarkers for detection of oral squamous cell carcinoma in a Taiwanese population. Clin Cancer Res. 2016;22:3340–7.

41. Gonçalves AS, Arantes DAC, Bernardes VF, Jaeger F, Silva JM, Silva TA, et al. Immunosuppressive mediators of oral squamous cell carcinoma in tumour samples and saliva. Hum Immunol. 2015;76:52–8.

42. Hamzavi M, Tadbir AA, Rezvani G, Ashraf J, Fattahi MJ, Khademi B, et al. Tissue Expression , Serum and Salivary Levels of IL-10 in Patients with Head and Neck Squamous Cell Carcinoma. Asian Pacific J Cancer Prev. 2013;14:1681–5.
43. Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, Iqbal J, et al. Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest. 2015;33:318–28.

44. Onuchic AC, Chammas R. Câncer e o microambiente tumoral. Rev Med. 2010;89:21.

45. Ikram N, Hassan K, Tufail S. Cytokines. Int J Pathol. 2004;2:47–58.

46. Curry JM, Sprandio J, Cognetti D, Luginbuhl A, Bar-ad V, Pribitkin E, et al. Tumor Microenvironment in Head and Neck Squamous Cell Carcinoma. Semin Oncol. 2014;41:217–34.

47. MANTOVANI A, SOZZANI S, LOCATI M, ALLAVENA P, SICA A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23:549–55.

48. Jiang C, Ye D, Qiu W, Zhang X, Zhang Z, He D, et al. Response of lymphocyte subsets and cytokines to Shenyang prescription in Sprague-Dawley rats with tongue squamous cell carcinomas induced by 4NQO. BMC Cancer. 2007;7:40.

49. Prasad G, McCullough M. Chemokines and cytokines as salivary biomarkers for the early diagnosis of oral cancer. Int J Dent. 2013;2013:813756.

50. SCIUBBA JJ. Oral cancer and its detection. J Am Dent Assoc. 2001;132:12S–18S.

51. Pereira LHM, Adebisi IN, Perez A, Wiebel M, Reis I, Duncan R, et al. Salivary markers and risk factor data: A multivariate modeling approach for head and neck squamous cell carcinoma detection. Cancer Biomarkers. 2011;10:241–9.

52. Osman TA, Costea DE, Johannessen AC. The use of salivary cytokines as a screening tool for oral squamous cell carcinoma: A review of the literature. J Oral Maxillofac Pathol. 2012;16:256–61.

53. Rezaei F, Mozaffari HR, Tavasoli E, Zavattaro E, Imani MM, Sadeghi M. Evaluation of Serum and Salivary Interleukin-6 and Interleukin-8 Levels in Oral Squamous Cell Carcinoma Patients: Systematic Review and Meta-Analysis. J Interferon Cytokine Res. 2019;jir.2019.0070.

| Table |
### Table 1. Salivary cytokines in oral cancer.

| Cytokine | Author | Year | Groups | n of cases | Mean ± SD (pg/ml) | Statistic test | p Value | AUC |
|----------|--------|------|--------|------------|-------------------|----------------|---------|-----|
| IL 8     | SAHEBJAMEE M. et al. | 2008 | Oral Cancer | 9 | 1093.7 ± 1089 | Kolmogorov-Smirnov Test and Mann-Whitney U | <0.05 | - |
|          |        |      |        | 9 | 700.7 ± 1031.5 |                |         |     |
| ELASHOFF, D. et al. | 2012 | Oral Cancer - cohort 4 | 36 | 2563 ± 2179 | Mann-Whitney U test and area under the curve (AUC) | <0.05 | 0.68 |
|          |        |        | Control - cohort 4 | 54 | 808 ± 1132 |                |         |     |
|          |        | Oral Cancer - cohort 5 | 31 | 2140 ± 2282 |                |         | <0.05 |
|          |        | Control - cohort 5 | 70 | 739 ± 1002 |                |         |     |
| ARELLANO-GARCIA, M.E. et al. | 2008 | Oral Cancer | 40 | 3347.7 ± 2929 | Student t-test, Pearson correlation coefficient; ROC curve and area under curve | With single-plex: 0.02 | 0.62 | (Sensitivity=87.5% Specificity=64.3%) |
|          |        |        | Oral Cancer | 42 | 759.4 ± 563 |                |         |     |
|          |        |        | Periodontitis | 10 | 818.82 ± 228.41 |                |         |     |
|          |        |        | Periodontitis Control | 10 | 589.24 ± 370.29 |                |         |     |
| TANN, W. et al. | 2007 | Oral cancer | 20 | 1252 ± 456 | T-test and area under curve | <0.00001 | 0.837 |
|          |        |        | Control | 20 | 577 ± 355 |                |         |     |
| KATAKURA A. et al. | 2007 | Oral Cancer | 19 | 720 | Two-tailed t-tests | <0.05 | - |
|          |        |        | Control | 20 | 250 |                |         |     |
| RAJKUMAR, K. et al. | 2014 | Oral Cancer | 100 | 1091.68 ± 167.12 | Shapiro-Wilk’s test, Kruskal-Wallis analysis, Mann-Whitney U test, Spearman rank test, Receiver operator characteristic (ROC) curve and area under curve | <0.05 | PML x OSCC=0.971 (95%CI=0.953 - 0.990; p<0.0001) |
|          |        |        | Premalignant | 100 | 650.39 ± 207.26 |                |         |     |
|          |        |        | Control | 100 | 349.56 ± 115.12 |                |         |     |
| CHENG, L.Y.S. et al. | 2014 | Oral Cancer | 5 | 1525.33 ± 1123.95 | Kruskal-wallis test Mann-Whitney U test (post hoc) | <0.001 | - |
|          |        |        | Chronic periodontitis | 21 | 738.79 ± 394 |                |         |     |
|          |        |        | Disease active - Oral lichen planus | 15 | 1328.37 ± 731.8 |                |         |     |
|          |        |        | Disease inactive - Oral lichen planus | 13 | 1083.09 ± 646.19 |                |         |     |
|          |        |        | Control | 21 | 890.83 ± 563.22 |                |         |     |
| Cytokine | Author | Year | Groups | Sample (n) | Mean ± SD (pg/ml) | Statistic test | P Value | AUC |
|---------|--------|------|--------|------------|------------------|---------------|---------|-----|
| IL-8    | PUNYANI, S.R.; SATHAWANE, R.S. | 2013 | Oral Cancer | 25 | 1718.61 ± 668.294 | Scheffe's analysis and two-tailed independent samples t test. | <0.001 | - |
|         |        |      | Oral Precancer and Oral leukoplakia | 25 | 299.513 ± 158.165 |                |         |     |
|         |        |      | Control | 25 | 210.096 ± 142.302 |                |         |     |
|         | LEE, L.T. et al. | 2018 | Oral Cancer | 41 | 2060.32 ± 1796.53 | Kolmogorov-Smirnov and Mann-Whitney | <0.001 | 0.783 (Sensitivity: 65.85%, Specificity: 79.17%, p=0.0002) |
|         |        |      | Control | 24 | 906.99 ± 833.43 |                |         |     |
|         | RODHUS, N.L. et al. | 2005b | Oral lichen planus | 13 | 2492 ± 664.7 | One-way ANOVA, Student-Newman-Keuls q-test; t-test. | <0.001 | - |
|         |        |      | Oral Cancer | 13 | 4082.8 ± 752.3 |                |         |     |
|         |        |      | Control | 13 | 1507.2 ± 398.5 |                |         |     |
|         | MAIE, A. R. et al. | 2004 | Oral Cancer | 32 | 720 | t-test, receiver operating characteristic (ROC) analyses | <0.001 | 0.978 (Sensitivity: 86%, Specificity: 97%) |
|         |        |      | Control | 32 | 250 |                |         |     |
|         | KHYANI, I.A.M. et al. | 2017 | Oral Cancer | 35 | 873.6 | Pearson Chi-Square test, one-way ANOVA test, Post Hoc Dunnet t-test | <0.001 | - |
|         |        |      | Control | 35 | 52.1 |                |         |     |
|         |        |      | Premalignant | 35 | 305.0 |                |         |     |
|         | RODHUS, N.L. et al. | 2005a | Oral Cancer | 13 | 3154.1 ± 1023.2 | Not described | <0.001 | - |
|         |        |      | Control | 13 | 1580.7 ± 789 |                |         |     |
|         |        |      | Premalignant | 13 | 1918.2 ± 899.1 |                |         |     |
|         | GLEBER-NETTO, F.O. et al. | 2016 | Oral Cancer | 60 | 283.75 ± 262.33 | ANOVA (Kruskal-Wallis Test); Wilcoxon Two - Sample test | <0.0001 | PMOD vs. Controls=0.467, OSCC vs. Controls=0.449, OSCC vs. PMOD=0.518 |
|         |        |      | Malignant potential injuries | 60 | 140.35 ± 155.13 |                |         |     |
|         |        |      | Control | 60 | 127.79 ± 110.84 |                |         |     |
| IL-6   | PANNEER SELVAM N. and SADAKSHARAM J. | 2015 | Oral Cancer | 25 | 132.9 ± 59.1 | ANOVA and Kruskal-Wallis | <0.001 | - |
|         |        |      | Control | 25 | 9.7 ± 12.8 |                |         |     |
|         |        |      | Leucoplakia | 25 | 43.0 ± 52.1 |                |         |     |
| Cytokine | Author | Year | Groups | Sample (n) | Mean ± SD (pg/ml) | Statistic test | P Value | AUC |
|---------|--------|------|--------|------------|-------------------|----------------|---------|-----|
| IL-6    | JURETIĆ M. et al. | 2013 | Oral Cancer | 19 | 0.707 ± 0.234 | Kruskal-Wallis and Mann-Whitney | <0.001 | - |
|         |        |      | Control | 19 | 0.002 ± 0.002 |                |         |     |
|         |        |      | Pre-malignant | 19 | 0.431 ± 0.217 |                |         |     |
| IL-6    | SAHEBJAMEE M. et al. | 2008 | Oral Cancer | 9 | 40.9 ± 79.5 | Kolmogorov-Smirnov Test and Mann-Whitney U | <0.05 | - |
|         |        |      | Control | 9 | 2.5 ± 1.3 |                |         |     |
| IL-6    | ZHANG S. et al. | 2017 | Oral Cancer | 40 | 4.8 ± 1.0 | Unpaired t-test | <0.01 | - |
|         |        |      | Control | 20 | 1.3 ± 0.05 |                |         |     |
| IL-6    | DINESHKUMAR T. et al. | 2011 | Oral Cancer | 100 | 178 ± 28.3 | Nonparametric Mann-Whitney U tests and ROC curve analysis | <0.05 | 0.9 (Sensitivity = 99.0% Specificity = 96.0%) |
|         |        |      | Control | 100 | 10.3 ± 6.7 |                |         |     |
|         |        |      | Pre-malignant | 50 | 35.3 ± 14.3 |                |         |     |
|         |        |      | Condition Premalignant | 50 | 38.3 ± 12.3 |                |         |     |
| IL-6    | KATAKURA A. et al. | 2007 | Oral Cancer | 19 | 86.5 | Two-tailed t-tests | <0.05 | - |
|         |        |      | Control | 20 | - |                |         |     |
| IL-6    | BAGAN, L. et al. | 2015 | Oral Cancer | 20 | 435.04 ± 142.15 | Kruskal-Wallis test, Mann-Whitney U test, Spearman's rank correlation coefficient | <0.01 | - |
|         |        |      | Control | 20 | 33.4 ± 38.95 |                |         |     |
|         |        |      | Proliferative verrucous leukoplakia | 20 | 151.59 ± 129.27 |                |         |     |
| IL-6    | SATO, J. et al. | 2010 | Oral Cancer | 29 | 20.1 ± 36.3 | Mann-Whitney U test, Wilcoxon rank sum test, Spearman correlation coefficient | <0.003 | - |
|         |        |      | Control | 19 | 0.6 ± 0.8 |                |         |     |
| IL-6    | KHYANI, I.A.M. et al. | 2017 | Oral Cancer | 35 | 61.2 | Pearson Chi-Square test, one-way ANOVA test, Post Hoc Dunnet t tes | <0.001 | - |
|         |        |      | Control | 35 | - |                |         |     |
|         |        |      | Pre-malignant | 35 | 217.8 |                |         |     |
| Cytokine | Author | Year | Groups | Sample (n) | Mean ± SD (pg/ml) | Statistic test | P Value | AUC |
|----------|--------|------|--------|-----------|------------------|----------------|---------|-----|
| IL-6     | RODHUS, N.L. et al. | 2005 a | Oral Cancer | 13 | 88.2 ± 43.2 | Not described | <0.001 | - |
|          |        |      | Control | 13 | 1.4 ± 1.0 |                |         |     |
|          |        |      | Pre-malignant | 13 | 70.8 ± 24.3 |                |         |     |
| IL-6     | CHENG, L.Y.S. et al. | 2014 | Oral Cancer | 5 | 178.41 ± 172.32 | Kruskal-wallis test Mann-Whitney U test (post hoc) | <0.001 | - |
|          |        |      | Chronic periodontitis | 21 | 5.85 ± 4.02 |                |         |     |
|          |        |      | Disease active - Oral lichen planus | 15 | 20.74 ± 22.28 |                |         |     |
|          |        |      | Disease inactive - Oral lichen planus | 13 | 8.06 ± 7.96 |                |         |     |
|          |        |      | Control | 21 | 4.92 ± 8.77 |                |         |     |
| IL-6     | LEE, L.T. et al. | 2018 | Oral Cancer | 41 | 198.33 ± 303.84 | Kolmogorov-Smirnov and Mann-Whitney | <0.001 | 0.823 (Sensitivity: 82.9% Specificity: 70.3% p<0.0001) |
|          |        |      | Control | 24 | 69.23 ± 271.96 |                |         |     |
| TNF-α    | BRAILO, V. et al. | 2012 | Oral Cancer | 28 | 129 ± 66.29 | Smirnoff Kolmogorof and Kruskal Wallis | <0.012 | - |
|          |        |      | Leukoplakia | 29 | 18 ± 5.19 |                |         |     |
|          |        |      | Control | 31 | 16 ± 3.91 |                |         |     |
| TNF-α    | RHODUS, N.L. et al. | 2005 b | Oral lichen planus | 13 | 148.12 ± 21.3 | One-way ANOVA, Student-Newman-Keuls q-test and teste-t | <0.0001 | - |
|          |        |      | Oral Cancer | 13 | 198.23 ± 47.68 |                |         |     |
|          |        |      | Control | 13 | 2.26 ± 0.72 |                |         |     |
| TNF-α    | JURETIĆ M. et al. | 2013 | Oral Cancer | 19 | 0.739 ± 0.176 | Kruskal-Wallis and Mann-Whitney | <0.001 | - |
|          |        |      | Control | 19 | 0.013 ± 0.033 |                |         |     |
|          |        |      | Premalignant | 19 | 0.601 ± 0.178 |                |         |     |
| TNF-α    | RODHUS, N.L. et al. | 2005 a | Oral Cancer | 13 | 28.9 ± 14.6 | Not described | < 0.01 | - |
|          |        |      | Premalignant | 13 | 10.5 ± 7.4 |                |         |     |
|          |        |      | Control | 13 | 3.0 ± 1.0 |                |         |     |
| TNF-α    | SAHEBJAMEE, M. et al. | 2008 | Oral Cancer | 9 | 35.2 ± 51.8 | One Sample Kolmogorov-Smirnov test and Mann-Whitney U | <0.05 | - |
|          |        |      | Control | 9 | 4.1 ± 2.1 |                |         |     |

| Cytokine | Author | Year | Groups | Sample (n) | Mean ± SD (pg/ml) | Statistic test | P Value | AUC |
|----------|--------|------|--------|-----------|------------------|----------------|---------|-----|
| TNF-α    | LEE, L.T. et al. | 2018 | Oral Cancer | 41 | 27.75 ± 30.94 | Kolmogorov-Smirnov and Mann-Whitney | <0.001 | 0.749 (Sensitivity: 39.02% Specificity: 100% p=0.0001) |
|          |        |      | Control | 24 | 8.6 ± 7.27 |                |         |     |
| TNF-α    | POLZ-DACEWICZ, M. et al. | 2016 | Oral Cancer | 78 | 23100 | Pearson Chi-Square tests. Mann-Whitney and Kruksal-Wallis | 0.00002 | - |
|          |        |      | Control | 40 | 11300 |                |         |     |
| TNF-α    | BRAILO, V. et al. | 2012 | Oral Cancer | 28 | 34 ± 21.58 | Smirnoff | 0.126 | - |
| Cytokine | Author | Year   | Groups                  | Sample (n) | Mean ± SD (pg/ml) | Statistic test                                                                 | P Value   | AUC  |
|----------|--------|--------|-------------------------|------------|-------------------|--------------------------------------------------------------------------------|-----------|------|
| IL-1β    | LEE, L.T. et al. | 2018   | Oral Cancer             | 41         | 391.43 ± 540.39   | Kolmogorov-Smirnov and Mann-Whitney                                            | 0.002     | AUC: 0.729 (Sensitivity= 69.98%; Specificity= 79.17%; p=0.0004) |
|          |        |        | Control                 | 24         | 132.53 ± 175.79   |                                                                                  |           |      |
|          | BRAILO, V. et al. | 2012   | Oral Cancer             | 28         | 906 ± 62.21       | Smirnoff Kolmogorov and Kruskal Wallis                                          | 0.000     |      |
|          |        |        | Leukoplakia             | 29         | 143 ± 54.74       |                                                                                  |           |      |
|          |        |        | Control                 | 31         | 354 ± 61.41       |                                                                                  |           |      |
| IL-10    | HAMZAVI, M. et al. | 2014   | Oral Cancer             | 30         | 11.79 ± 10.66     | Kolmogorov-Smirnov test. Mann-Whitney, Kruskal-Wallis and Chi-Square tests       | 0.619     |      |
|          |        |        | Control                 | 24         | 10.01 ± 6.01      |                                                                                  |           |      |
|          | AZIZ, S. et al. | 2015   | Oral Cancer             | 30         | 4.45 ± 4.29       | Teste t. One way ANOVA e LSD Post hoc                                            | 0.004     |      |
|          |        |        | Control                 | 33         | 1.72 ± 1.33       |                                                                                  |           |      |
|          | LEE, L.T. et al. | 2018   | Oral Cancer             | 41         | 14.91 ± 20.21     | Kolmogorov-Smirnov and Mann-Whitney                                             | 0.355     |      |
|          |        |        | Control                 | 24         | 9.86 ± 8.5        |                                                                                  |           |      |
| Cytokine | Author | Year | Groups | Sample (n) | Mean ± SD (pg/ml) | Statistic test | P Value | AUC   |
|----------|--------|------|--------|------------|------------------|----------------|---------|-------|
| IL-1     | RODHUS, N.L. et al. | 2005 a | Oral Cancer | 13 | 454.4 ± 215.8 | Not described | < 0.01 | -     |
|          |        |      | Premalignant | 13 | 255.1 ± 124.8 |                |         |       |
|          |        |      | Control | 13 | 173.2 ± 66.9 |                |         |       |
| IL-1RA   | AZIZ, S. et al. | 2015 | Oral Cancer | 30 | 2831.69 ± 2506.41 | Teste - t. One way ANOVA e LSD Post hoc | 0.096 | -     |
|          |        |      | Control | 33 | 1949.2 ± 1178.02 |                |         |       |
| IL-4     | AZIZ, S. et al. | 2015 | Oral Cancer | 30 | 1.17 ± 1.96 | Teste - t. One way ANOVA e LSD Post hoc | 0.589 | -     |
|          |        |      | Control | 33 | 1.01 ± 0.8 |                |         |       |
| IL-13    | AZIZ, S. et al. | 2015 | Oral Cancer | 30 | 0.76 ± 0.57 | Teste - t. One way ANOVA e LSD Post hoc | 0.01 | -     |
|          |        |      | Control | 33 | 0.23 ± 0.19 |                |         |       |

**Figures**
Figure 1

PRISMA flow diagram of study selection process (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009)
Figure 2
Sample size distribution among the investigated studies according to: A) Salivary cytokines assessed. IL-8, IL-6, TNFα and IL-1β were the cytokines investigated in the biggest number of patients; B) Patient clinical groups (OC = Oral Cancer; Control = healthy individuals; PMOD = Potentially Malignant Oral Disorders, OLP = Oral Lichen Planus). Healthy patients were the most numerous among control groups, followed by individuals with PMOD; C) Salivary cytokines assessed per each patient clinical group.

**Figure 3**

Salivary cytokine expression ratio among clinical control groups related to oral cancer group. Cytokine expression ratios were calculated by dividing the mean salivary cytokine expression levels of control groups (healthy controls; oral lichen planus [OLP] controls, gingivitis controls and potentially malignant oral lesions [PMOL] controls) by the mean expression levels in oral cancer groups for each individual study (Y-axis). Values below the red line (Y-axis = 1) indicate that oral cancer patients had higher cytokine expression levels than their respective controls, while values above the red line indicate that oral cancer patients had lower cytokine expression levels than their respective controls.
Figure 4
Distribution of salivary cytokine expression ratios among the different analyzed studies. Cytokine expression ratios were calculated by dividing the mean salivary cytokine expression levels of control groups (healthy controls; oral lichen planus [OLP] controls, gingivitis controls and potentially malignant oral lesions [PMOL] controls) by the mean expression levels in oral cancer groups for each individual study (Y-axis). Values to the left of the red line (X-axis = 1) indicate that oral cancer patients had higher cytokine expression levels than their respective controls, while values to the right of the red line indicate that oral cancer patients had lower cytokine expression levels than their respective controls.