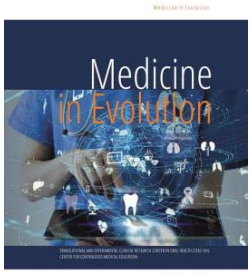
Salivary interleukin-6, interleukin-8, and Tumor Necrosis Factor-alpha as a potential biomarker panel for early detection of oral squamous cell carcinoma



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Abstract

Aim: Salivary components are now receiving more attention as potential biomarkers for various diseases. This study aims to investigate the possibility of using IL -6, IL -8 and TNF-α as salivary biomarkers for early detection of OSCC

Methods: This study followed the PRISMA protocol for reviews and meta-analyses and used PubMed as a database to identify articles that examined salivary concentrations of these mediators as potential biomarkers for early detection of oral squamous cell carcinoma.

Results: We found 14 studies that examined salivary markers in oral pathology and met the requirements for review.

Conclusion: Most studies showed increased concentrations of these mediators in the saliva of patients with OSCC. However, the values of salivary concentrations of IL -6, IL -8, and TNF-α in both OSCC and healthy patients remain controversial. Moreover, salivary concentrations of these mediators are influenced by the presence of oral comorbidities or the use of mouthwashes.

Keywords: Interleukin, tumor necrosis factor, oral squamous cell carcinomas

INTRODUCTION

Saliva, along with blood, is one of the body fluids that contains a number of components whose properties can be helpful in diagnosing and evaluating the effectiveness of treatments for various diseases (1). Saliva contains proteins, metabolites, hormones, mRNA and enzymes (2). Previous studies have shown that these are present in saliva in much higher concentrations than in blood (3). The simple and non-invasive way of collection makes saliva a suitable tool for the detection of specific biomarkers (4). Analysing variations in their concentrations or changes in their structure/function in saliva is a simple way to diagnose and monitor certain diseases, with the detection of various diseases in their early stages or the identification of patients at risk of developing chronic diseases or even cancer being essential (5).The extent to which these goals are achieved can have a critical impact on the treatment, progression, and prognosis of these diseases, which in turn can affect the well-being, quality of life, and even lifespan of patients suffering from these diseases. The discovery of specific salivary biomarkers that provide information contributing to these goals is critical to the dynamics of this process.

Considering the multifactorial elements associated with the etiopathogenesis of oral cancers, the most recent studies focused on assessing a panel of salivary biomarkers targeting proteomic and transcriptomic targets (2, 5, 6, 7). Although most recent studies use modern techniques for detecting a multi-target panel with implications in oral squamous cell carcinoma (OSCC) pathogenesis, there is no uniformity in the results. Moreover, the same salivary biomarkers are also found in other cancers, such as ovarian, lung, and pancreatic cancer (8, 9, 10). In addition, the concentration of salivary biomarkers varies with salivary flow, viscosity, and dietary intake (11).

In recent years, mounting evidence has suggested that cytokines play a significant role in carcinogenesis (12). In most tumor processes, there is an abundant cytokine palette of pro-inflammatory cytokines, chemokine, and growth factors associated with tumor induction and development (13). Most neoplastic processes show a perturbance in the balance between cell survival and apoptosis (14). Among the principal cytokines involved in this process are the pro-inflammatory cytokines interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) and chemokine interleukin-8 (IL-8) (15). Data from studies so far suggest that IL-6, IL-8, and TNF-α contribute to the initiation of the neoplastic process by protecting it from apoptosis and favoring cell growth and angiogenesis (16). These inflammatory mediators have been detected in tumor cells and stroma but also in the plasma and saliva of patients with oral and general diseases, including cancer (14,15, 19).

Aim and objectives

In this study, I aim to analyze the potential usefulness of a combination of IL- 6, IL- 8, and TNF-α as a reliable salivary panel biomarker for early detection of oral cancer.

METHODOLOGY

An electronic literature search for research articles published between 2003 and 2023 was conducted using PubMed. For the search engine, the following keywords were used:

OSCC, oral premalignant lesion, oral leukoplakia, dental caries, gingivitis, periodontitis combined with salivary interleukin, or salivary cytokine.

Study selection

Study selection was done using the PRISMA statement. Study selection was conducted using the following criteria.

Inclusion criteria

1. Original research articles including interleukin 6, 8, and TNF-alfa as a salivary biomarker panel in oral diseases and disorders.
2. Original research articles including over ten subjects.
3. Full text is available.

Exclusion criteria

1. Reviews, meta-analysis, case series.
2. Studies that did not analyze this biomarker panel in saliva.
3. Studies that analyze this biomarker panel implication in cancer progression, evolution, metastasis, and survival rate.
4. Studies including subjects less than eighteen years old.

Abstracts were exported to Sci-Hub for full text.

Data extraction

From the included articles, we extract only data referring to the interleukin 6, interleukin 8, and TNF-alpha.

RESULTS

The initial search of the PubMed database identified 597 articles. After evaluating the articles, we included 14 articles in this analysis (Figure. 1).

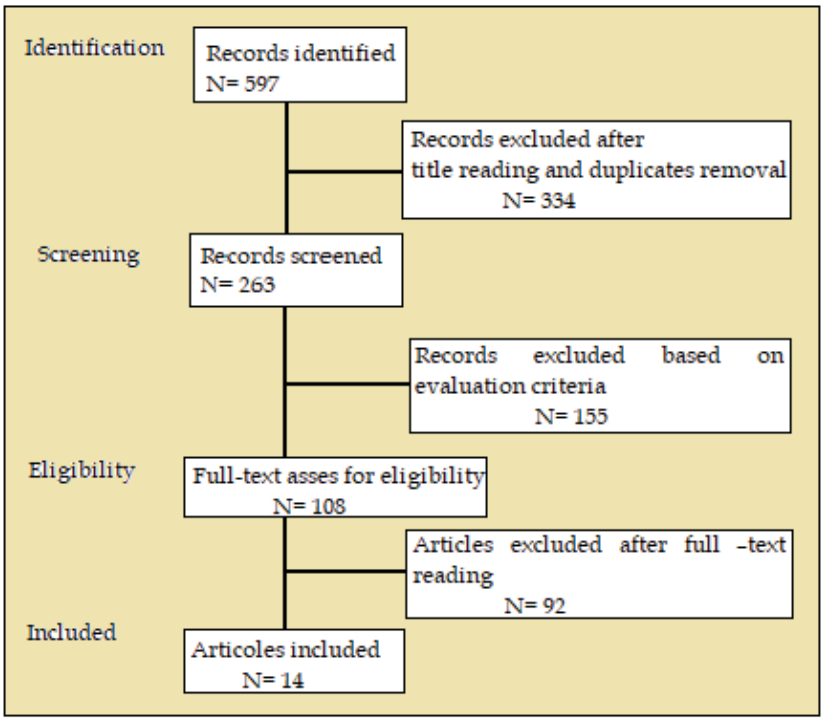

Figure 1. PRISMA flowchart of study selection

DISCUSSIONS

Squamous cell carcinoma is the most common oral tumor (17), and diagnosis at advanced stages challenges treatment efficacy, prognosis, and survival. Despite all efforts, there are currently no biomarkers for early diagnosis of OSCC or for identifying patients at increased risk of developing OSCC. However, there are a number of indications of the

possibility of using inflammatory mediators for the detection of early OSCC (16). Because of its accessibility, the noninvasive nature of saliva collection (18), and also because of its direct contact with the tumor process, it is considered a suitable tool for the detection of these mediators (16). Among the inflammatory mediators, IL -6, IL -8, and TNF-α, are among the most studied salivary compounds in OSCC and other oral diseases and disorders.

Previous data from studies examining this salivary panel suggest that these mediators are significantly elevated in the saliva of OSCC patients in most cases. Lee et al. (2017), Csősz et al. (2017), Dikova et al. (2021), and Rai et al. (2021) found increased levels of IL -6, IL -8, TNF-α in the saliva of OSCC patients compared to patients without cancer using the same study method (Luminex-based multiplex kit) (Table 1) (3,19,20, 21). Moreover, in the analysis performed by Dikova et al. a progressive increase in the levels of these cytokines in the saliva of patients with early-stage OSCC was detected compared to patients with oral leukoplakia (OL) and healthy patients (Table 2), indicating the pre-neoplastic character of OL (21).

Table 1. Salivary cytokine in OSCC

Study	Citokine	p-value	Diseases evaluated
Lee at al. 2017 (3)	**IL-6 ↑** **IL-8 ↑** **TNF-α ↑**	**< 0.001** **0.001** **0.001**	**OSCC > HC**
Csősz et al. 2017 (19)	**IL-6 ↑** **IL-8 ↑** **TNF-α ↑**	**0.0002** 0.1087 **0.0157**	**OSCC > HC**
Dikova et al. 2021 (21)	**IL-6 ↑** **IL-8 ↑** **TNF-α ↑**	**<0.001** **<0.001** **<0.001**	**OSCC > HC** **OSCC > HC** **OSCC > HC**
Rai et al. 2020 (20)	**IL-6 ↑** **IL-8 ↑** **TNF-α↑**	**0.0259** **0.0228** **0.0321**	**OSCC > HC**

Abbreviation: OSCC, oral squamous cell carcinoma; HC, Healthy control; ↑, Increased level

Similar results were previously reported by Rhodus et al (2005), who used ELISA analysis to detect elevated levels of these mediators in the saliva of patients with OSCC and premalignant oral lesions compared with controls (Table 2) (22).

Table 2. Salivary cytokine in OSCC versus oral precancerous lesions

Study	Citokine	p-value	Diseases evaluated
Dikova et al. 2021 (21)	**IL-6 ↑** **IL-8 ↑** **TNF-α ↑**	**a) <0.001; b) 0.001** **a)≤0.05; b)0.004** **a)<0.001; b)0.001**	**a)OSCC> OL** **b)OL>HC**
Rhodus et al. 2005 (22)	**IL-6 ↑** **IL-8 ↑** **TNF-α↑**	**<0.001** **<0.001** **<0.01**	**OSCC> OPML>HC**

Abbreviation: OSCC, oral squamous cell carcinoma; OL, oral leucoplakia: HC, Healthy control; OPML, oral premalignant lesion; ↑, Increased level

Salivary levels of this group of mediators were also examined independently of OSCC. Using an ELISA assay, Kaur et al. (2015) found significantly elevated levels of these cytokines in the saliva of patients with premalignant oral lesions compared with controls (23). In this study, subgingival fibrosis had the highest salivary levels of IL -6, IL -8, and TNF-alpha (Table 3). In contrast, in a 2022 study examining these three mediators, only IL -6 and TNF-α were found to be significantly elevated in the saliva of patients with oral lichen planus (OLP) (24).

Table 3. Salivary cytokine in oral precancerous lesio versus healthy control

Study	Citokine	p-value	Diseases evaluated
Kaur et al. 2015 (23)	**IL-6 ↑**	**<0.05**	**SB>OL>OLP>HC**
	IL-8 ↑	**<0.05**	**SB>OLP>OL>HC**
	TNF-α ↑	**<0.05**	**SB>OLP>OL>HC**
Zhu et al. 2022 (24)	**IL-6 ↑**	**0.022**	**OLP > HC**
	IL-8 ↑	0.172	
	TNF-α ↑	**0.012**	

Abbreviation: OLP, Oral lichen planus; OL, oral leucoplakia; HC, Healthy control; SB, submucous fibrosis; ↑, Increased level

Aside from OSCC and salivary premalignant lesions, IL-6, IL-8, and TNF-α, have been considered salivary biomarkers for inflammatory processes in the oral cavity. Evaluation of the salivary concentration of these mediators showed a significant increase of these mediators in the saliva of patients with dental caries (25, 26). Salivary levels of IL -6, IL -8 and TNF-α were also significantly increased in patients with chronic periodontitis (Table 4) (27).

Table 4. Salivary cytokine in oral inflammatory condition

Study	Citokine	p-value	Diseases evaluated
Gornowicz et al. 2012 (25)	**IL-6 ↑**	**<0.005**	**DC>HC**
	IL-8 ↑	**<0.008**	
	TNF-α ↑	**<0.002**	
Hussein et al. 2020 (26)	**IL-6 ↑**	**0.005**	**DC>HC**
	IL-8 ↑	**0.008**	
	TNF-α ↑	0.063	
Kaczyński et al. 2019 (27)	**IL-6 ↑**	**< 0.0001**	**PG>HC**
	IL-8 ↑	**< 0.0001**	
	TNF-α ↑	**< 0.0001**	

Abbreviation: DC, dental caries; HC, Healthy control; PG, periodontitis group; ↑, Increased level

Following the salivary values of IL-6, IL-8, and TNF- α detected in the studies analyzed, we observed significant differences between the salivary concentrations of these mediators in healthy patients. Salivary concentrations can vary up to 2.5-fold for TNF- α, 5-fold for IL-6 and 6-fold for IL-8 (Table 5).

In addition, Rhodus et al. determined a TNF-α level in the saliva of patients with OSCC (28.9 ± 14.6 pg/ml) that was lower than the level found by Gornowicz et al. in patients with dental caries (36.50 ± 41.46 pg/ml), using the same examination method (22, 25). Controversy regarding salivary concentrations in OSCC was also noted for Il-8. Korrostof determined values of 1242 ± 408 pg/ml in exophytic lingual SCC, 1585 ± 348 pg/ml in healthy smoking patients, and 1672 ± 310 pg/ml in healthy patients consuming alcohol and smoking (28).

Table 5. Saliva concentrations of IL-6, IL-8, and TNF- α in healthy people were measured using the ELISA technique. (pg/ml)

Study	IL-6	IL-8	TNF-α
Laliberte at al. 2021 (29)	5.21 ± 1.14	256.50 ± 86.21	3.06 ± 0.66
Dikova et al. 2021 (21)	7.95 ± 0.95	526.17± 59.03	7.62 ± 0.84
Gornowicz et al. 2012 (25)	2.68 ± 5.51	619.19 ± 311.79	7.32 ± 6.98
Korrostof et al. 2011 (28)	3.4 ± 1	932 ± 262	3.9 ± 2.6
Rhodus et al. 2005 (30	2.18 ± 0.71	1507.2 ± 398.5	3.36 ± 2.07
Rhodus et al. 2005 (22)	1.4 ± 0.9	1580 ± 789.0	3.0 ± 1.9

In addition, the oral localization of OSCC and the associated periodontal inflammation caused by poor oral hygiene in these patients lead to changes in salivary concentrations of these cytokines (3). In addition, the use of mouthwash for a prolonged period of time was found to affect salivary concentrations of IL -6, IL -8, and TNF-α. OLP patients who used

mouthwash for one month showed a decrease in the concentration of these mediators according to Rhodus et al. (2006) (31).

CONCLUSIONS

Despite promising results showing remarkable differences of these mediators in OSCC compared with premalignant lesions or healthy patients, there are still conflicting results regarding the concentrations of these mediators in saliva. To date, there is no clear consensus on the concentration of these mediators in saliva of healthy individuals. In addition, the concentration of these mediators in saliva may be influenced by the presence of local comorbidities or the use of mouth rinses.

Although these salivary biomarkers have been detected in OSCC, the evidence is not convincing enough to consider the combination of IL -6, IL -8, and TNF-α as a valid salivary biomarker panel for early detection of OSCC or for screening high-risk patients.

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