# Oral Squamous Cell Carcinoma Biomarkers

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Oral cancers are among the most common malignant tumors worldwide. More than 90% of all oral malignancies are oral squamous cell carcinomas (OSCC). While in Western countries, OSCC accounts for about 4% of all cancers; in India and Southeast Asia, it reaches up to 40%. The interaction between oral dysplastic/neoplastic cells and saliva makes this fluid a source of biomarkers, such as cytokines.

Keywords: oral cancer ; oral squamous cell carcinoma ; biomarkers ; cytokines ; oral potentially malignant disorders

## 1. Introduction

The clinical course of OSCC is strictly related to the time of diagnosis [1]. According to the Surveillance, Epidemiology, and End Results (SEER) Program (based on the U. S. population), when OSCC is confined to the primary site, the 5-years relative survival rate is about 85.1%, decreasing to 66.8% and to 40.1% for tumors spread to regional lymph nodes and to distant sites, respectively [2].

Classical risk factors for OSCC development in Western countries are smoking and alcohol abuse. The role of some human papillomaviruses (HPV) infection has been clearly established for carcinomas of the oropharynx, but doubts still exist on the oncogenic potential of such viruses in the rest of the oral cavity [3].

As for the rest of the head and neck tumors, the pathogenesis of OSCC is still a matter of controversy. Most authors agree on a multi-step process in which the accumulation of genetic and epigenetic changes affect protein expression, thus altering a variety of signaling pathways [4]. Gene alterations may range from the gain or loss of single nucleotides, up to partial or complete deletion of chromosomes. The loss of genes has been described in significant conjunction with inactivating mutations of tumor suppressor genes [5].

In a recent review on the molecular landscape in head and neck cancer, Leemans et al. identified five cellular processes that may be dysregulated in OSCC pathogenesis: cell cycle, growth signal, survival, WNT signaling and epigenetic regulation [6].

Diagnosis of OSCC is based on clinical inspection followed by biopsy and histopathological evaluation of suspicious tissues. Vital staining (e.g., toluidine blue) and auto-fluorescence imaging may highlight tissues undergoing rapid cell division and represent complementary diagnostic aids, as they can differentiate normal from dysplastic or cancerous tissues and direct the biopsy to the target site [7]. Radiographic imaging is used to investigate the involvement of the surrounding tissue and structures, such as muscles, bone, and lymph nodes; in particular, computed tomography (CT) and magnetic resonance imaging (MRI) are essential for bone and neck nodes evaluation [8].

Some OSCCs are preceded by oral potentially malignant disorders (OPMDs), including leukoplakia, erythroplakia, lichen planus and oral submucous fibrosis [9][10], at the histological level.

Visual examination alone, however, may lead to ignoring subtle lesions and failing to differentiate malignant from benign oral conditions. As a result, OSCC is diagnosed at an already advanced stage, adversely affecting the survival rate. Many studies are, therefore, focused on the identification of easily accessible early diagnostic biomarkers.

## 2. Salivary Cytokines as Biomarkers for Oral Squamous Cell Carcinoma

It is well accepted that inflammation and cell-mediated immunity are active players in the control of oral carcinogenesis progression [11]. Cells' evasion from immune surveillance has been described as a primary step in oncogenesis [12]. Pro-inflammatory cytokines (IL-6, IL-8, TNF-α, soluble interleukin-2 receptor (sIL-2R)) are key-molecules involved in the

crosstalk between stromal and cancer cells, their expression being, to some extent, associated with tumor growth promotion or inhibition [13].

Serum levels of pro-inflammatory growth factors and cytokines in patients with OSCC or other oral potentially malignant disorders are still poorly investigated. Recent results would demonstrate that the serum level of IL-6, IL-8 and sIL-2R is significantly higher in patients with OSCC compared to healthy controls and to patients with OPMDs [11]. Thus, it appears feasible to accept that some salivary cytokines might be considered reliable biomarkers for OSCC diagnosis.

All the selected studies, investigating the role of specific salivary cytokines in the diagnosis of OSCC, identify the institutional care facility that enrolled the OSCC patients and, in most cases, mention the approval of an institutional ethical committee, thus presenting appropriate information about ethical protection.

As for saliva collection and processing, we ascertained, overall, a reasonable procedural consistency. In fact, most of the studies (1) analyze unstimulated whole saliva, obtained via passive drool; (2) include a preliminary step of centrifugation at 4 °C to separate cells and debris; (3) freeze the saliva supernatant, usually at −80 °C, until the analysis. Nevertheless, only in a few studies did the salivary protein content, including cytokine molecules, appear to be protected by the addition of protease inhibitors [14][15][16][17][18].

In all studies, an enzyme-linked immunosorbent assay (ELISA), based on antibodies against specific cytokines and a colorimetric revelation system, turned out to be the method of choice for cytokine quantitation. The method allows the accurate detection of the antigen, but measures only one cytokine in each sample, with consequent sample waste and high cost when the purpose is to measure multiple cytokines. In four included studies that investigated the potential variation in the concentration of several cytokines, a multiplex bead-based immunoassay was preferred [19][14][20][21]. Besides the high throughput multiplex analysis, the major advantage is its broader dynamic range of cytokine concentration measurable as compared to the ELISA test.

Since the possible presence of dental and periodontal infection may influence salivary cytokine concentrations [22], many of the selected studies evaluate the periodontal status of the study participants. While most of them simply exclude subjects with periodontitis from control and/or patient groups or include control subjects with periodontal status matched with the patient group, the studies of Brailo and co-workers [23] and Sato and co-workers [24][25] take into account the periodontal status of all the study participants. Thus, by using the community periodontal index, as described by the World Health Organization (WHO), they can demonstrate that the salivary concentrations of IL-6 and TNF-α are not affected by periodontal health.

In addition, except for a few cases [15][26][16][27], the authors agree on excluding subjects with any relevant comorbidity, for example, chronic or acute illnesses, autoimmune diseases, chronic inflammatory diseases, pathological dry mouth syndrome, or the inability to collect a sufficient saliva sample. We believe that the application of this exclusion criterion constitutes good practice, consistent with the diagnostic purposes of this topic area.

The interfering behavioral habits considered are mainly alcohol consumption and tobacco chewing or smoking. The use of these substances is frequent in OSCC patients, although preferential habits appear to be associated with the specific geographical origin of the patients. In only nine studies [19][14][23][28][27][29][30][31][25], the authors tested whether the salivary cytokine level was affected by social habits. As far as IL-1α, IL-1β, IL-6, TNF-α and IL-8 are individually concerned, their salivary concentrations did not appear to be affected by (or correlated to) specific habits.

In thirteen articles, salivary cytokine level is also assessed in the OPMD patient group and compared with OSCC and control groups. OPMD patients suffer prevalently from leucoplakia, but also from oral sub mucous fibrosis or oral lichen planus, while erythroplakia did not appear in any study, possibly because of its rare occurrence. In twelve studies, the salivary levels of IL-6, IL-8, and TNF-α of OPMD patients show a statistically significant higher concentration when compared with the control, but lower when compared to OSCC patients [19][32][29][33][34][30][35][36]. Likewise, comparable findings are obtained with IL-6 and TNF-α salivary concentrations [23][28] when compared to control subjects. In these cases, the salivary cytokines concentration allows us to differentiate between OPMD, OSCC and healthy subjects, thus reflecting a multi-diagnostic potential and corroborating the use of selected cytokines as biomarkers.

Among the studies including OPMD subjects, six articles investigated the diagnostic utility of the salivary cytokine level by ROC curve analysis and compared the ability of selected cytokines (IL-6, IL-8, TNF-α) to differentiate between patients with OSCC and OPMD [19][17][29][33][34][30]. The significant area under the curve (AUC) ranges from 0.70 to 0.99, reflecting an effective power of discrimination.

As observed in some studies [19][17][31], the combination of different salivary biomarkers is of great value for OSCC detection. A predictive model based on six cytokines and used for distinguishing OSCC from control subjects yielded a sensible increase in AUC as compared to individual cytokine analysis [19]. Additionally, the combination of cytokine proteomic and transcriptomic biomarkers generated an increased discriminatory effect between OSSC and control subjects [17][31], although further inclusion of a risk factor exposure (areca nut, smoking) provided the best panel of variables useful for OSCC detection [17].

Unfortunately, in OSCC patients, treatment outcome and prognosis are seriously affected not only by late diagnosis but also by frequent loco regional recurrences. Sato and co-workers [24][25] perceived the potential of IL-6 in terms of recurrence prediction and demonstrated that the sequential analysis of salivary cytokines post-treatment could be a useful marker for the diagnosis of early and late loco regional recurrence, a common cause of mortality in patients with OSCC. To give one example, they measured an increased postoperative IL-6 concentration in subjects with early recurrence as compared to subjects without recurrence. Apparently, if validated with a larger number of patients enrolled prospectively, the analysis of the dynamic behavior of that cytokine level in the post-treatment phase might be an effective tool for the early identification/prediction of recurrence.

According to these observations, we also expect an appropriate combination of biomarkers to be validated in longitudinal studies, and their reliability to be confirmed with respect to potential confounding factors such as behavioral habits and periodontitis.

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