Salivary Biomarkers for Oral Squamous Cell Carcinoma: a meta-analysis

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a malignant neoplasm with complex etiopathogenesis and an overall 5-year survival rate below 60% attributed to advanced disease stage at diagnosis. Saliva is increasingly being investigated as a non-invasive diagnostic tool for screening and follow-up of OSCC, while the need for a reliable, clinically applicable salivary biomarker for early diagnosis of OSCC remains unmet. The purpose of the present study was to identify potentially significant salivary biomarkers for OSCC.

Methods: PubMed and Google Scholar databases were searched using specified keywords to identify and shortlist relevant studies. The included studies were assessed using the Newcastle-Ottawa Scale (NOS) for quality. Meta function in Stata version 12.0 was used to perform meta-analysis.

Results: Out of 82 studies identified using PubMed and Google Scholar databases, 11 were included in our meta-analysis. Three markers (IL-8, IL-6, and Albumin) were analyzed in more than one study and all three markers had significantly higher mean protein expression in OSCC than healthy controls.

Conclusion: IL-8, IL-6, and Albumin are all potential, non-invasive salivary biomarkers for OSCC with significantly elevated mean protein expression in the saliva of OSCC patients compared to healthy controls.

Keywords: Oral cancer, Saliva Biomarker, Protein OSCC, IL-8, IL-6, Albumin

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a malignant neoplasm that originates from the mucosa lining oral cavity as well as the oropharynx and constitutes 2-3% of global cancer burden with increasing incidence recorded over past several decades[1-3]. The disease is associated with significant morbidity and mortality with an overall 5-year survival rate bordering short of 60% (4, 5). The prognosis varies significantly with tumor stage and cases diagnosed earlier are associated with up to 80% survival rate in contrast to 30-50% for cases that are diagnosed at an advanced stage, warranting judicious search for early biomarkers that will potentially aid in prompt screening and diagnosis of patients with early invasive malignancies[6, 7].

OSCC has a complex, multifactorial etiopathogenesis with the majority of the cases...
following a period of dysplasia that may or may not manifest itself clinically as a potentially malignant lesion (PML) with widely variable (0-20% in 1-30 years) rate of malignant transformation. Histopathological assessment of these PMLs remains the current gold standard for confirming or ruling out invasive malignancies, a time-consuming, invasive, and expensive process that is ill-suited for widespread screening (8).

In recent years, recognizing the unmet need for laboratory diagnostic tools for early detection of OSCC, plenty of research has been carried out to investigate saliva for its potential use as a non-invasive diagnostic tool owing to its direct contact with oral tissues and OSCC lesions (9). The process of collecting saliva is easy, cost-effective, free from contamination risk, and non-traumatic to the patient, thus it is a promising alternative to serum testing and biopsy (8). Since the late 1990s, several scientific literatures have been published investigating over 100 various salivary constituents including metabolites, proteins, and nucleic acids as potential salivary biomarkers for OSCC (4). Despite ongoing research, the need for a reliable, clinically applicable salivary biomarker for early diagnosis of OSCC remains uncharted (10).

The purpose of the present study was to identify potentially significant salivary biomarkers for OSCC.

** METHODOLOGY**

Present meta-analysis has been performed in accordance with the guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

**Search protocol**

PubMed and Google Scholar databases were searched using the following keywords: (‘oscc’ or ‘oral squamous cell carcinoma’) and (‘saliva’) and (‘protein’) and (‘biomarkers’). The endpoint of the search was 2018.

**Study selection and selection criteria**

Only studies in the English language were selected. The studies were included in the analysis if (1) they were case-control studies; (2) they reported mean (± standard deviation [SD]) of salivary protein biomarker levels; (3) the controls were healthy and without OSCC; and (4) there were no restrictions with regard to gender and age.

**Quality assessment**

The quality of the studies included in the meta-analysis was assessed using the Newcastle-Ottawa Scale (NOS). The total score of 7 or greater was considered to be of high quality (11).

**Statistical analyses**

Meta function in Stata version 12.0 was used to perform a meta-analysis. The choice of random and fixed effect model was based on a test of heterogeneity. If $I^2$ was more than 50% with its p-value less than 0.05 then the random effect model was built else fixed-effect method was used.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Marker</th>
<th>Mean marker in control</th>
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<td>28.80</td>
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<td></td>
<td></td>
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<tr>
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<td>S100P</td>
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<td>35.38</td>
<td>4.44</td>
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Table 1: Characteristics of the studies included in meta-analyses (N=11) *(IL-8 – Interleukin -8, IL-6- Interleukin-6, SAT1-Spermidine/spermine N(1)-acyetyltransferase 1, OAZ1-Ornithine decarboxylase antizyme 1, DUSP1-Dual specificity protein phosphatase 1, S100P-S100 calcium-binding protein P, H3F3A- Histone H3.3, MMP-9 – Matrix metallopeptidase 9, 8OHD-8-Oxo-2'-deoxyguanosine, MDA-Malondialdehyde , CEA-Carcinoembryonic antigen , ErbB-2- Receptor Tyrosine Kinase 2 , BFGF-Basic Fibroblast Growth Factor , CA 125 – Cancer Antigen 125)*
RESULTS

Out of 82 studies identified using PubMed and Google Scholar databases and screened, 36 studies were reviewed after the removal of irrelevant and duplicate studies. Out of these 36 studies, 11 studies were included in meta-analysis after the exclusion of 25 studies for various reasons (Figure 1). The characteristics of the 11 studies included in the meta-analysis are documented in Table I. All studies in total encompassed 339 OSCC cases and 346 controls.

IL-8 levels in saliva

Three studies reported IL-8 levels in saliva included 103 patients and 106 controls. Two of them showed a moderate effect of IL-8 on OSCC. One study showed a 3 times higher effect on OSCC by IL-8 levels in saliva. Figure 2 shows the effect size of IL-8 salivary levels in patients with OSCC compared with controls. The combined effect elucidated IL-8 produced a 1.5 times higher effect on OSCC as compared to controls (P=0.02).

IL-6 levels in saliva

Figure 3 shows the SMD of IL-6 salivary levels in patients with OSCC compared with controls. Only two studies were found fulfilling the criteria included 46 controls and 43 OSCC patients. The study from Paneer Selvam et al presented a twice higher risk of OSCC due to IL-6 as compared to study from Cheng et al. The combined effect revealed 2.17 times higher significant effect of OSCC as compared to the control group (P=0.002).

Albumin levels in saliva

With 45 cases and 45 controls from two studies extracted from literature, it was observed that albumin salivary levels elicit a significant effect on OSCC. The results were nearly concurrent in both the studies. Heterogeneity was also less than 50%, (P=0.876), hence the fixed-effect model was used to execute meta-analysis for this marker. As displayed in figure 4, the combined effect depicted 1.72 times
higher chances of having OSCC as compared to control (P<0.0001).

<table>
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<th>Comparability (score)</th>
<th>Exposure/Outcome (score)</th>
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<td>Honarmand (2016)</td>
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**Table 2:** Quality assessment of included studies on the basis of NOS (N=11)

*Figures and text*:

*Figure 1:* Flowchart of the study. * 4 were review articles, 8 did not study protein markers, 4 were studies on oral premalignant lesions, 4 studies were based on identification techniques, and 5 studies were excluded for not reporting mean*
Fig. 2: Forest plot showing SMD in IL-8 levels in saliva.

Fig. 3: Forest plot showing SMD in IL-6 levels in saliva.
Salam et al., Salivary Biomarkers for Oral Squamous Cell Carcinoma: a meta-analysis

DISCUSSION

A biomarker is defined as “any substance, structure, or process that can be measured in the body and influence or predict the incidence or outcome of disease”\(^{(12)}\). Biomarkers are variable substances whose levels in tissues and body fluids, including saliva alter in diseased versus healthy states, thereby allowing their quantification to serve as a tool for diagnosis and follow-up of affected individuals\(^{(13)}\). A wide array of transcriptomic, genomic and proteomic markers have been investigated in the saliva of OSCC patients against healthy controls including, but not limited to, various inflammatory cytokines, growth factors and their receptors, and various enzymes\(^{(10}, 14-16\)). Of these molecules, the cytokine group has been a target of primary interest, because of its pivotal role in inducing and regulating cellular communications. Their aberrant expression can be an indicator of tissue pathological processes, including cancer \(^{(5}, 7, 10\)).

The present meta-analysis also showed cytokine, Interleukin-8 (IL-8) to be the most frequently investigated marker amongst included studies. In 3 separate case-control studies conducted in Taiwan, the USA, and India conflicting results emerged with Gleber-Netto et al. \(^{(4)}\) reporting insignificant difference in mean expression of IL-8 between OSCC cases and controls, whereas Lisa Cheng\(^{(17)}\) and Punyani \(^{(18)}\) reported significantly higher expression of IL-8 in cases of OSCC. Studies conducted in the USA and India by Lisa Cheng\(^{(17)}\) and Paneer Selvam \(^{(19)}\), respectively, reported a significant difference in mean values of IL-6 between cases and controls. Our meta-analysis reported an SMD of 1.26 pg/mL for IL-8 with a significance of \(P=0.000\) and for IL-6 the SMD was 2.17 pg/mL with a significance of \(P=0.011\).

Tumor cells utilize a multitude of cellular processes and pathways to survive and multiply. Reactive oxygen species (ROS) such as superoxide anion are highly reactive free radicals derived as oxygen metabolism byproducts. Cancer cells harbor elevated levels of ROS which in turn is associated with initiation, progression, and chemoresistance of malignant tumors \(^{(20}, 21\)). To maintain physiological homeostasis, scavenging mechanisms exist to maintain redox balance. These antioxidant systems include enzymes such as catalase, and various non-enzymatic substances such as ascorbic acid \(^{(20}, 22\)).

![Fig. 4: Forest plot showing SMD in Albumin levels in saliva.](image-url)
serum and extracellular proteins also exhibit antioxidant properties by virtue of their free thiol groups. Among them, albumin is recognized as a potent antioxidant with abundant availability and presence in saliva to counteract oxidative stress (23, 24). Two independent studies conducted in India by Rao Koduru (25) and Metgud (22) have reported significant differences in the mean protein expression of Albumin in OSCC cases compared to controls. Our meta-analysis yielded SMD value for Albumin at 1.717 g/dL identifying albumin as a potential biomarker. Cytokines and albumin levels in saliva, however may also be elevated in medically compromised patients and in patients inflicted with other inflammatory disease states of oral cavity (18).

Conclusion:

IL-8, IL-6, and Albumin are all potential, non-invasive salivary biomarkers for OSCC with significantly elevated mean protein expression in the saliva of OSCC patients compared to healthy controls. A limitation of the present study was a thin number of studies and a lack of longitudinal studies with follow-up of marker levels with treatment. More large-scale studies are needed with long-term follow-up.

Conflict of interest:

All authors do not have any conflict of interest.

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References

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