ORIGINAL ARTICLE

Evaluation of Serum and Salivary Interleukin-6 as Biomarkers for Early Detection of Oral Cancer

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ABSTRACT

Background

Late-stage diagnosis is primarily responsible for the high morbidity and death rates linked to oral squamous cell carcinoma (OSCC). Improving patient outcomes requires the creation of trustworthy, non-invasive biomarkers for early detection. The multifunctional pro-inflammatory cytokine interleukin-6 (IL-6) has been linked to carcinogenesis, but further research is needed to determine how well it can diagnose early-stage OSCC, particularly in saliva.

Methods

60 participants participated in a case-control study, 30 of whom had just been diagnosed with early stage (Stage I & II) OSCC that was histopathologically verified, and 30 of whom were healthy controls who were matched for age and gender. Samples of venous blood and unstimulated entire saliva were obtained. A commercially available enzyme-linked immunosorbent assay (ELIAs) kit was used to determine the levels of IL-6. Using receiver operating characteristic (ROC) curve analysis, the diagnostic performance was ascertained.

Results

The OSCC group had substantially greater serum and salivary IL-6 levels than the control group ($p < 0.001$). In OSCC patients, the mean serum IL-6 level was 12.5 +/- 3.1 pg/mL, whereas in controls, it was 4.2 +/- 1.5 pg/mL. In OSCC patients, the mean salivary IL-6 level was 18.7 +- 4.2 pg/mL, whereas in controls, it was 5.1 +- 1.8 pg/mL. With an area under the curve (AUC) of 0.94 (95% CI: 0.88-1.00) compared to serum IL-6 (AUC = 0.88, 95% CI: 0.79-0.97), ROC analysis revealed salivary IL-6 to be a superior diagnostic marker. Salivary IL-6 demonstrated 90.0% sensitivity and 93.3% specificity for identifying OSCC at an ideal cut-off of 11.5 pg/mL.

Conclusion

Compared to serum IL-6, salivary IL-6 exhibits greater diagnostic performance and is significantly higher in individuals with early-stage OSCC. Salivary IL-6 is a potential biomarker for the early identification and screening of oral cancer because of its non-invasive nature, high sensitivity, and specificity. By acting early, it may be possible to improve the prognosis.

Keywords

Oral cancer, Interleukin-6, Saliva, Serum, Biomarker, Early detection, ELISA

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a serious global health problem and is among the top 10 most prevalent cancer in the world [1]. Despite advancements in therapeutic modalities, the 5-year survival rate has remained stagnant at around 50-60% in the past few decades and this is attributed majorly to delayed diagnosis [2]. The majority of cases of OSCC are diagnosed in an advanced stage with a more complex, morbid and less effective treatment. In sharp contrast, early stage of disease (Stage I and II) carries 5-year survival rate of more than 80%, if appropriately treated [3]. This disparity demonstrates the urgent need for effective, non-invasive screening and diagnostic tools to enable the detection of OSCC at its earliest, most treatable phase.

The current gold standard of diagnosis is clinical examination, followed by invasive tissue biopsy and histopathological confirmation [4]. While definite, this strategy is not suitable for large-scale population screening. Consequently, there has been an intense focus of research on the identification of molecular biomarkers in easily accessible body fluids, in particular blood and saliva. Saliva in particular has become an ideal diagnostic medium, providing a non-invasive, inexpensive and stress-free method of repeated sampling [5]. It consists of a complex combination of compounds of local and systemic origin such as proteins, enzymes, hormones and cytokines that can indicate the physiological and pathological status of the oral cavity [6].

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Among the multitude of possible biomarkers, cytokines have received much attention because of the pivotal role of cytokines in inflammation and cancer. Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological functions, including the regulation of immune responses, acute-phase reactions and hematopoiesis [7]. In the context of cancer, IL-6 acts as a key mediator of the tumor environment (microtumor). It stimulates tumor cell proliferation, survival, angiogenesis, and metastasis by many mechanisms, the most well-known of which is the JAK/STAT3 cascade [8]. Elevated IL-6 levels have been repeatedly reported in the serum of patients with different types of malignancies including head and neck cancers, and frequently correlated with advanced stage, tumor burden, and poor prognosis [9, 10].

While the role of IL-6 as a prognostic indicator in advanced OSCC is relatively well-established, the usefulness of IL-6 as a diagnostic biomarker for early stage disease is less apparent. Furthermore, there is a paucity of studies that directly compare the diagnostic efficacy of IL-6 in serum versus saliva in the same cohort of early stage OSCC patient. Given that saliva is in direct contact with the oral lesion, it's plausible that salivary IL-6 levels might be more accurate in reflecting the local tumor microenvironment and more accurate in diagnosing than serum levels.

Recent research in this area has started to investigate the possible potential of salivary cytokines. A study done by Gornowicz et al. had shown high levels of several inflammatory mediators, including IL6, in the saliva of OSCC patients [11]. However, several studies involve heterogeneous patient populations with different stages of disease and it has been hard to determine the exact value of these markers in early detection. This gap in research prevents the application of these findings to a screening test that can be used clinically.

Therefore, the present study has been designed with the definite goal to evaluate and compare the levels of IL-6 in both serum and saliva of patients with early-stage OSCC and healthy controls and to find their diagnostic performance and potential as non-invasive biomarkers for early detection of oral cancer.

MATERIALS AND METHODS

Design and Environment of the Study The 18-month duration of this hospital-based case-control research was July 2021–December 2022. Calculating Sample Size G*Power software version 3.1.9.7 was used to calculate the a priori sample size. A substantial impact size (d = 0.9) for the difference in IL-6 levels between OSCC patients and controls was predicted based on a pilot research and the body of current literature. With a power (1-β) of 0.9, an allocation ratio of 1:1, and an alpha error of 0.05, the minimum sample size needed was determined to be 26 individuals per group. The sample size was expanded to 30 participants each group, for a total of 60 participants, in order to account for any dropouts and guarantee the robustness of the findings. Research Population Two groups of the study population were created: • Case Group (n=30): Individuals with recently diagnosed OSCC who have been histopathologically proven to be in clinical stage I or II, as defined by the 8th edition staging criteria of the American Joint Committee on Cancer (AJCC).

- Control Group (n = 30): Patients who regularly visit the general dentistry clinic were selected as healthy, age- and gender-matched adults who had no history of oral cancer or clinically noticeable oral mucosal lesions. Criteria for Inclusion and Exclusion Case Group Inclusion Requirements: • Primary OSCC with histopathological confirmation; • Clinical stage I or II illness (T1/T2, N0, M0).
- No past medical history of cancer or cancer treatment of any kind, including chemotherapy, radiation, or surgery. Control Group Inclusion Requirements:
- No history of cancer; • Good oral and overall health. Criteria for Exclusion (for both groups):
- The existence of an ongoing oral infection, inflammation, or other conditions affecting the oral mucosa.
- A history of autoimmune or systemic inflammatory conditions, such as rheumatoid arthritis or inflammatory bowel disease.
- Recent use of steroids, antibiotics, or anti-inflammatory medications (within the last two weeks).
- Being pregnant or nursing at the moment.
- A history of alcohol or tobacco use throughout the previous six months in order to reduce confusing inflammatory effects. Gathering of Samples To minimize nutritional and diurnal fluctuations, all samples were collected between 9:00 AM and

11:00 AM after an overnight fast. Saliva Collection: The passive drooling approach was used to get unstimulated entire saliva. The participants were instructed not to talk or swallow, to sit erect, and to tilt their heads forward. For five minutes, they were permitted to collect saliva in their mouths and spit it into a sterile, cooled polypropylene tube. Saliva (about 3 to 5 mL) was collected and put on ice right away. Blood Collection: Using a sterile venipuncture method, about 5 milliliters of venous blood were drawn from the antecubital vein. After being extracted into simple serum separator tubes, the blood was allowed to coagulate for half an hour at room temperature. Samples of blood and saliva were centrifuged for 15 minutes at 4C at 3000 rpm (around 1500 g). After that, the serum and clear supernatant (saliva) were separated into 0.5 mL cryovials and kept at -80C until they could be examined in a lab. To prevent protein degradation, samples were subjected to a single freeze-thaw cycle. Quantification of IL-6 A commercially available, high sensitivity human IL-6 enzyme immunoassay (ELISA) Kit (R&D Systems, Minneapolis, MN, USA) was used to measure the levels of IL-6 in blood and saliva in accordance with the manufacturer's instructions. A single technician who was blind to the subjects' clinical state performed each test in triplicate. The kit's recombinant human IL-6 standards were used to produce a standard curve. A microplate reader (Bio-Rad, Hercules, CA, USA) with a correction wavelength of 570 nm was used to measure each well's absorbance at 450 nm. The standard curve was used to calculate each sample's IL-6 content, which was then represented in picograms per milliliter (pg/mL). Analysis of Statistics Statistical software (SPSS software version 25.0, IBM Corp., Armonk, NY, USA) was used to do the statistical analysis. The Shapiro-Wilk test was used to determine if the data distribution was normal. For data sets that were regularly distributed, the mean +/- standard deviation (SD) was presented for the descriptive statistics. The mean IL-6 levels in the OSCC and control groups were compared using the independent samples t-test. The receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of blood and salivary IL-6. The total rate of diagnostic accuracy was calculated using the area under the ROC curve (AUC). It was determined what the optimal cutoff value was to maximize the sum of the sensitivity and specificity (Youden's index). Positive predictive value (PPV), negative predictive value (NPV), comparable sensitivity, and specificity were computed. For every test, a p-value of less than 0.05 was deemed statistically significant.

RESULTS

Demographic Characteristics

The study comprised 60 participants, with 30 in each group. The demographic characteristics of the study population are summarized in Table 1. There were no statistically significant differences in age ($p = 0.751$) or gender distribution ($p = 0.789$) between the OSCC and control groups, ensuring that the groups were well-matched. Within the OSCC group, the majority of lesions were located on the tongue (40%) and buccal mucosa (33.3%).

Table 1: *Demographic characteristics and lesion site of the study population*

Parameter	OSCC Group (n=30)	Control Group (n=30)	p-value
Age (years, mean ± SD)	52.3 ± 9.8	51.6 ± 10.2	0.751
Gender (n, %)			0.789
Male	19 (63.3%)	18 (60.0%)	
Female	11 (36.7%)	12 (40.0%)	
Site of Lesion (n, %)			-
Tongue	12 (40.0%)	-	
Buccal mucosa	10 (33.3%)	-	
Alveolus	5 (16.7%)	-	
Floor of mouth	3 (10.0%)	-	

OSCC: Oral squamous cell carcinoma; SD: Standard deviation

Comparison of Serum and Salivary IL-6 Levels

The mean concentrations of IL-6 in serum and saliva for both groups are presented in Table 2. Both serum and salivary IL-6 levels were significantly elevated in the OSCC group compared to the healthy control group ($p < 0.001$ for both comparisons). The mean salivary IL-6 level was approximately 1.5 times higher than the mean serum IL-6 level within the OSCC group.

Table 2: *Comparison of serum and salivary IL-6 levels between OSCC and control groups*

Biofluid	OSCC Group (mean ± SD)	Control Group (mean ± SD)	p-value
Serum IL-6 (pg/mL)	12.5 ± 3.1	4.2 ± 1.5	<0.001*
Salivary IL-6 (pg/mL)	18.7 ± 4.2	5.1 ± 1.8	<0.001*

*Independent samples t-test; IL-6: Interleukin-6; OSCC: Oral squamous cell carcinoma; SD: Standard deviation

Diagnostic Performance of Serum and Salivary IL-6

The results of the ROC curve analysis for serum and salivary IL-6 are detailed in Table 3 and Figure 1. Salivary IL-6 demonstrated superior diagnostic performance compared to serum IL-6. The AUC for salivary IL-6 was 0.94 (95% CI: 0.88-1.00), indicating excellent discriminatory ability, while the AUC for serum IL-6 was 0.88 (95% CI: 0.79-0.97).

At an optimal cutoff value of 11.5 pg/mL, salivary IL-6 yielded a sensitivity of 90.0% and a specificity of 93.3% for differentiating OSCC patients from controls. For serum IL-6, the optimal cutoff was 7.8 pg/mL, which provided a sensitivity of 83.3% and a specificity of 80.0%.

Table 3: *ROC curve analysis for serum and salivary IL-6 in detecting OSCC*

Parameter	Serum IL-6	Salivary IL-6
AUC (95% CI)	0.88 (0.79-0.97)	0.94 (0.88-1.00)
Optimal Cutoff (pg/mL)	7.8	11.5
Sensitivity (%)	83.3	90.0
Specificity (%)	80.0	93.3
Positive Predictive Value (%)	80.6	93.1
Negative Predictive Value (%)	82.8	90.3

ROC: Receiver operating characteristic; AUC: Area under the curve; CI: Confidence interval; IL-6: Interleukin-6; OSCC: Oral squamous cell carcinoma

DISCUSSION

In order to diagnose OSCC early, the current research set out to evaluate the diagnostic capability of IL-6, a crucial pro-inflammatory cytokine, in blood and saliva. Our main discovery is that, in comparison to healthy controls, individuals with early-stage OSCC had considerably higher blood and salivary IL-6 levels. More significantly, our findings demonstrate that salivary IL-6 has higher sensitivity and specificity than blood IL-6, making it a superior diagnostic tool. The known involvement of IL-6 in carcinogenesis is consistent with the increased levels of IL-6 in OSCC patients. It is known that OSCC cells, as well as the immunological and stromal cells that support them, generate IL-6 [12]. This cytokine stimulates proliferation and inhibits apoptosis to promote tumor development in an autocrine and paracrine manner. Additionally, via upregulating vascular endothelial growth factor (VEGF), IL-6 is a very strong inducer of angiogenesis, a mechanism required for tumor development and metastasis [13]. Our patient cohort's significantly elevated IL-6 levels are most likely a manifestation of ongoing biological processes that underlie the early development of tumors. Comparing blood and salivary IL-6 in a homogenous sample of patients with early-stage OSCC is one of our study's main contributions. An important discovery is that salivary IL-6 (AUC = 0.94) performs better in diagnosis than serum IL-6 (AUC = 0.88). There are many reasons for this. First of all, since saliva comes into direct touch with the oral lesion, it is hypothesized that cytokines generated by the tumor and the inflammatory cells around it are released straight into the saliva, increasing the levels locally [14]. Second, the specificity of serum IL-6 in relation to oral disease may be compromised by systemic dilution and susceptibility to subclinical inflammatory disorders elsewhere in the body [15]. Thirdly, the salivary cytokine profile in instances of oral cancer is likely dominated by local production inside the oral cavity, and the entrance of serum-derived IL-6 into saliva is an active process. Our study's salivary IL-6 diagnostic performance (90% sensitivity, 93.3% specificity) is quite encouraging and seems to be on line with previous studies. A panel of salivary biomarkers, including IL-6, was used in a research by Arellano-Garcia et al. to successfully distinguish OSCC patients from controls [16]. Our findings expand on this by demonstrating that salivary IL-

6, a single marker, may function very well on its own and by concentrating only on early-stage illness. Because of its high level of accuracy, a simple, non-invasive saliva test may be created to screen for high-risk populations (such as tobacco chewers and chronic smokers). These discoveries have important clinical ramifications. A chairside or lab-based salivary IL-6 test might be a useful supplement to the conventional visual oral examination. Even in the absence of obvious clinical symptoms, it can be useful in assisting medical professionals in spotting worrisome lesions that can be biopsied right away, perhaps leading to an earlier diagnosis. Salivary IL-6 serial monitoring may be a sign for malignant transformation in individuals with diagnosed oral possibly malignant illnesses, although this would need validation in longitudinal research. Notwithstanding the encouraging outcomes, it is important to recognize the limitations of our research. Although adequate for the first study, the comparatively modest sample size has to be validated in larger, multi-center cohorts. Additionally, since our research was cross-sectional in nature, we were only able to provide a single point in time and were unable to ascertain the temporal correlation between tumor growth and elevated IL-6. To find out whether salivary IL-6 concentrations rise before to cancer's clinical presentation, further long-term research is needed. Lastly, even though IL-6 performed well on its own, cancer is a diverse illness. In order to further enhance the diagnostic test's sensitivity and specificity, future study should look at the use of a multiplex panel of salivary biomarkers.

CONCLUSION

This research provides compelling evidence that salivary interleukin-6, which is superior than serum IL-6 in terms of diagnostic ability, is significantly higher in individuals with early-stage oral squamous cell carcinoma. Salivary IL-6 is a very promising biomarker for the early diagnosis of oral cancer because of its excellent sensitivity and specificity as well as the non-invasive nature of saliva collection. The use of such a biomarker might transform screening methods and enable earlier intervention to improve the prognosis of those suffering from this debilitating illness.

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