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Investigation of salivary interleukin-17 in patients with oral squamous cell carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a common aggressive cancer with few noninvasive biomarkers for early detection. A-17, a pro-inflammatory cytokine involved in tumor progression could be used as a salivary biomarker for the diagnosis and monitoring of OSCC. **Objectives**: To measure and compare levels of IL-17 in saliva of OSCC patients and healthy controls as well as to evaluate the expression of IL-17 in relation to tumor location and patient gender. Methods: In the Medical City Complex, Baghdad, Iraq a case-control study was carried out between March 2024 and January 2025. In total, 90 participants were enrolled; patients and controls matched for age and gender with 55 OSCC patients and 35 healthy controls. Clinically and histopathologically confirmed OSCC. Unstimulated saliva samples were collected under standardized conditions and analyzed for IL-17 concentration by using a commercially available ELISA kit. Statistical analyses were also carried out to compare IL-17 levels between groups as well as associations with tumor location and gender. **Results**: The OSCC patients had significantly higher salivary IL-17 levels (86.22 ± 19.56 pg/mL) as compared to healthy controls (44.12 ± 13.24 pg/mL) ($T = 5.87$, $p = 0.022$). Tumor location showed a significant association with the values of IL-17 ($F = 9.12$, $p = 0.004$). The highest values were for tumors in buccal mucosa, gingiva, and tongue; the lowest was for tumors in the palate. No significant difference was noted between males and females regarding IL-17 levels ($p=0.11$). **Conclusion**: High levels of salivary IL-17 are linked to OSCC and its level changes with the site of the tumor lesion, which suggests IL-17 as a good easy test for finding and watching OSCC. Being male or female does not seem to affect IL-17 levels.

KEYWORDS

Interleukin-17, oral lesions, OSCC, gingiva, palate

INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes a major portion of head and neck squamous cell carcinoma (HNSCC); it is aggressive and etiologically linked to the risk factors tobacco usage and human papillomavirus (HPV) infection. (Johnson et al., 2020). It is common knowledge that OSCC has a global prevalence and a poor prognosis, mostly attributed to late-stage diagnoses (Markopoulos, 2012). Lifestyle factors relate to the progression of the disease and include tobacco use; however, lesions do not present at the onset of the condition. Since these are risk factors, that understanding is essential. Early detection leads to much better treatment outcomes. Research has highlighted the need for timely interventions which can be implemented once potentially malignant disorders are identified (Machiels et al., 2020).

The present studies have advanced the futuristic diagnostic ability. Salivary metabolomics can be considered an upcoming non-invasive diagnostic tool since major metabolites in the saliva show significant changes between healthy and OSCC individuals (Sridharan et al., 2019). The above-presented facts prove early diagnosis capability via metabolic profiling besides

tracking tumor evolution. Also, further exploration into long non-coding RNAs (lncRNAs) has unlocked their aptitude to act as biomarkers for detecting OSCC, thus leading to novel paths in the realm of non-invasive diagnostics (Sasahira & Kirita, 2018).

Another reason for paying more attention to the phenotypic changes of tumor-associated macrophages (TAMs) in cancer is that it may open the door for new therapies (Tang et al., 2013). Therefore, the real challenge remains how to push these biomarkers into clinical practice for better prognostic capabilities in OSCC (Shaban et al., 2019). Surgery has long been established as an important part of multimodal treatment along with chemoradiotherapy and targeted therapy in modern approaches to be applied on patients with OSCC. Besides being described as a cornerstone for the treatment of this disease, surgical intervention has also been associated with improved survival outcomes when used for more advanced stages of this malignancy (Greene et al., 2019). This study further supports individualized treatment plans according to tumor characteristics, which is aligned with a current best practice recommendation (Lin et al., 2022).

The interleukins most relevant to OSCC are 6, 8 and TNF-α, which have been greatly studied for their action in this pathology. These cytokines act in increased levels with tumor growth, angiogenesis and metastasis. For example, according to Sahibzada et al (2017), salivary IL-6, IL-8 and TNF-α might be diagnostic biomarkers for oral cancer; therefore these cytokines can be applied as non-invasive indicators of any early detection of changes in OSCC. Costa et al (2013) addressed tumor-associated macrophages and presented an inflammatory cytokine profile that includes IL-10 which modulate immune responses that lead to OSCC invasion. The other interleukin important in the context of the microenvironment in OSCC is IL-1β. Wu et al., (2017) illustrated NLRP3 inflammasome mediation regarding chemoresistance against OSCC with high expression of IL-1β being indicative of a poor prognosis; therefore targeting both might improve therapeutic treatment.

It has not been well studied the role of Il-17 in the pathophysiology and diagnostic purposes in patients with OSCC. Though it is increasingly perceived that IL-17 is related to OSCC, many gaps in knowledge remain about it. First, the exact cellular sources of IL-17 in the tumor microenvironment of OSCC are not well delineated; therefore, immune cells that produce IL-17 dominantly in OSCC should be identified to inform targeted therapeutic approaches. Second, mechanistic pathways whereby elevated levels of IL-17 associate with poor prognosis need more illumination since studies have made such a correlation. Longitudinal studies are needed to explore causal relationships between IL-17 levels and tumor progression as well as patient outcomes. This study tries to assess salivary levels of Interleukin-17 (IL-17) in patients with OSCC.

PATIENTS AND METHODS

The current case-control study was carried out at the oncology center, in the Medical City, Baghdad, Iraq. The study duration extended from March 2024 to January 2025; thus, the enrollment of 90 participants who were divided into two groups: 55 patients diagnosed with oral squamous cell carcinoma (OSCC) and 35 healthy controls. Clinical evaluation complemented by histopathological findings from special oral pathologists was used to establish a diagnosis of OSCC. Age, gender, and relevant medical history were obtained by a standardized questionnaire and review of the medical record. This information was used in matching cases and controls and to assess possible confounding variables. Unstimulated total saliva samples were taken from all subjects under standard conditions, preferably in the morning at least 90 minutes post food or drink intake to reduce variability. Samples spun at 3000 rpm for 10 minutes with resulting supernatant kept at –80°C up to biochemical analysis.

Salivary interleukin-17 (IL-17) levels quantified by a commercially available enzyme-linked immunosorbent assay (ELISA) kit per the protocol of the manufacturer. Consistency and accuracy of results ensured by analyzing all samples in duplicate.

Ethical Considerations

The study was carried out following the principles of the Declaration of Helsinki. Ethical approval has been obtained from the Institutional Review Board (IRB) at Medical City Complex. All participants were informed about the purpose and procedures of the study and gave written informed consent to participate in the study. Personal and medical information confidentiality was strictly maintained throughout the study.

Statistical analysis

Data were analyzed by IBM SPSS Statistics version 25. Descriptive statistics summarized demographic and clinical characteristics. Age and salivary IL-17 levels are expressed as means ± standard deviation; gender and the group classification (OSCC vs. control) are presented as frequencies and percentages. Comparison of mean salivary IL-17 levels in OSCC patients and healthy controls was done using a one-way analysis of variance (ANOVA). For comparisons involving more than two subgroups (location of tumor), ANOVA accompanied by post hoc testing (for example, Tukey's test) was applied to determine significant pairwise differences. The Chi-square (χ^2) test was used to check the association between categorical variables; for example, the gender distribution in the two study groups. A value of $p < 0.05$ was considered statistically significant in all analyses (Al-Fahham, 2018).

RESULTS

The demographic distribution of the participants demonstrated no statistically significant difference between OSCC patients and healthy controls regarding age and gender. The greater percentage of participants in both groups fell within the age bracket of 35-44 years. The gender distribution was near-balanced; however, there was a slight female predominance in both groups. Chi-square analysis gave P-values of 0.78 for age and 0.89 for gender, both being NS, meaning that the two groups are demographically well-matched. This therefore provides a good balance for making comparisons in terms of salivary IL-17 levels between the two groups without major confounding by age or gender differences (Table 1).

Table 1. Age and gender distribution of both OSCC patients and control

Indicators		`Patients (No. = 55)		Control (No. = 35)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-34	12	21.82	9	25.71	0.06	0.78 (NS)
	35-44	22	40.00	16	45.71		
	≥ 45	21	38.18	10	28.57		
Gender	Male	26	47.27	17	48.57	0.011	0.89 (NS)
	Female	29	52.73	18	51.43		

The tumor lesion locations among patients with OSCC show the site to be most frequently affected on the buccal mucosa in 41.8% cases followed by the tongue in 38.2%. Lesions of the palate and gingiva were comparatively rare making up 10.9% and 9.1% respectively, this pattern speaks to surface areas of the oral cavity that are exposed quite frequently to carcinogenic risk factors e.g., tobacco and betel nut which seem to have a more intense effect on the buccal mucosa as well as the tongue, clinically relevant for tumor localization for early detection planning together with targeted interventions.

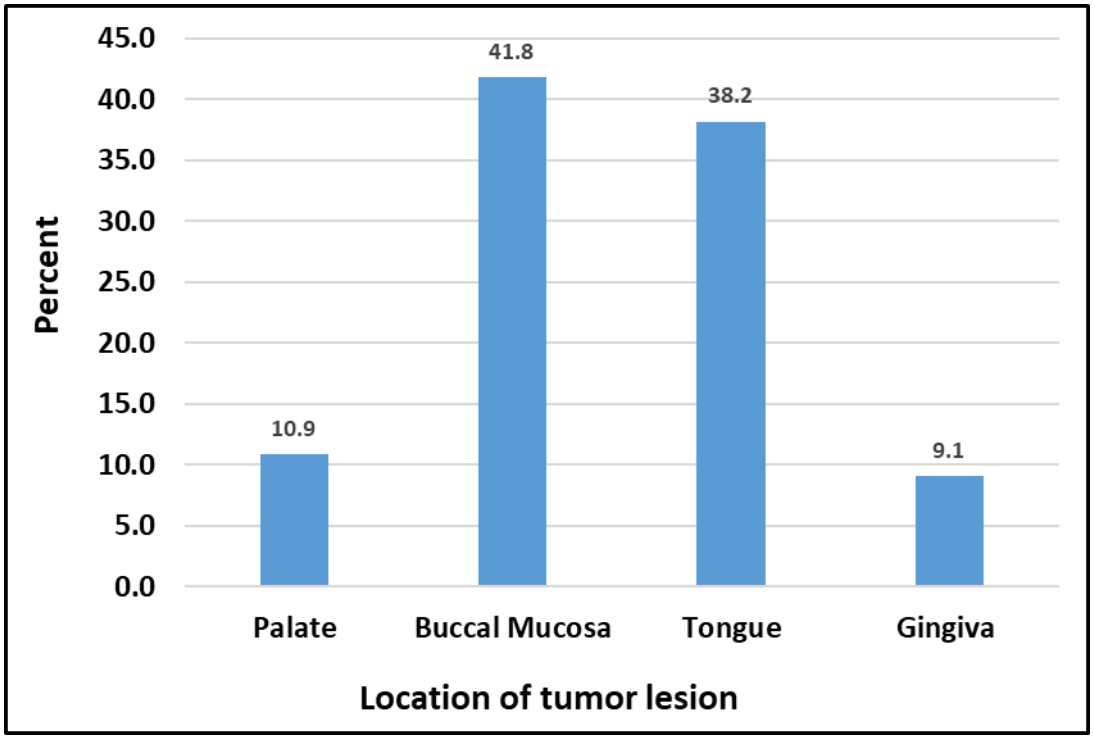

Figure 1. Distribution of patients with OSCC according to location of lesion

Comparison of salivary IL-17 levels in OSCC patients and healthy controls showed a statistically significant elevation in the patient group. The mean IL-17 concentration in OSCC patients was 86.22 ± 19.56 pg/mL, well above that in the control group which was 44.12 ± 13.24 pg/mL. Control vs Patient T value (5.87) P value (0.022) Elevated salivary IL-17 may be associated with the presence of OSCC and can potentially be used as an easier biomarker for disease detection or monitoring (Table 2).

Table 2. Assessment of IL-17 levels between OSCC patients and control participants

Groups	**No.**	**IL-17 (pg/ml)** **Mean ± SD**	**T Test** **(P Value)**
Patient	55	86.22 ± 19.56	5.87 (0.022)
Control	35	44.12 ± 13.24	

Table 3 gives the evaluation of IL-17 levels (pg/ml) in patients according to where the tumor is located; it shows major differences between groups. The top IL-17 levels were seen in the cheek mucosa group (95.3 ± 12.11), next was the gums (88.2 ± 11.12) and tongue (86.6 ± 11.12); all three are marked with a "B" which means there is no big difference between them. However, the palate group had much lower IL-17 levels (73.7 ± 13.19), marked as "A". The analysis gave a statistically significant difference between groups (F test = 9.12, p = 0.004); it also showed that where the tumor is located is linked to changes in how much IL-17 is expressed. The notation "HS" refers to a highly significant difference <0 .01.

Table 3. Assessment of IL-17 levels in patients' groups according to location of tumor

Groups	Freq.	IL-17 (pg/ml) Mean ± S.D	F test	T test P-value
Palate	6	A 73.7 ± 13.19	**9.12**	**0.004 (HS)**
Buccal Mucosa	23	B 95.3 ± 12.11		
Tongue	21	B 86.6 ± 11.12		
Gingiva	5	B 88.2± 11.12		

A, B Different letters refer to significant difference at p <0.05

Table 4 shows a comparison of IL-17 levels in male and female patients. Mean levels in males are slightly higher than females (88.37 ± 14.68 pg/ml vs. 83.44 ± 14.56 pg/ml). The difference is not statistically significant as indicated by the T test value of 1.69 and a p-value of 0.11, NS (not significant). In other words, gender does not significantly influence the expression levels of IL-17 in the patient population under study.

Table 4. Assessment of IL-17 levels in patients' groups according to gender

Groups	Freq.	IL-17 (pg/ml) Mean ± SD	T test P-value
Male	26	88.37 ± 14.68	**1.69 (0.11) NS**
Female	29	83.44 ± 14.56	

DISCUSSION

Results of the present study indicated that elevated salivary IL-17 may be associated with the presence of OSCC and can potentially be used as an easier biomarker for disease detection or monitoring. Recent studies have shown the involvement of IL-17 in the pathogenesis of tumors, including OSCC. High levels of IL-17 are found to be associated with tumor progression and metastasis. Interleukin-17 is a product mostly produced by Th17 cells and plays a very complex role in immunological reactions and inflammation. This has been involved not only chronic inflammatory diseases but also in mechanization of tumors (McGeachy et al., 2019). In the case of OSCC, high levels of IL-17 can promote a pro-tumorigenic microenvironment by increasing angiogenesis plus immune evasion (Blauvelt & Chiricozzi, 2018). The inflammatory ways activated by IL-17 may help tumor growth and so keep on making a cycle of inflammation that holds tumors growing larger (Jin & Dong, 2013).

Increased levels of IL-17 have been reported in OSCC patients in several studies. It is presumed to relate high concentrations of this interleukin with increased tumor growth and metastasis (Mills, 2022; Kuwabara et al., 2017). In the dysregulated tumor microenvironment, IL-17 responses can be skewed to facilitate further tumor progression and immune evasion; therefore, this interleukin actually bridges two functions by driving both

inflammation and providing a niche that supports malignancy (Gelderblom et al., 2012). Besides, the expression of other proinflammatory cytokines like IL-12 and IL-23 related to this one highlights it as a central player in chronic inflammation seen with OSCC (Moschen et al., 2018).

Studies have fairly explained how IL-17 affects the pathogenesis of OSCC through various mechanisms. It has been suggested that IL-17 promotes PD-L1 expression in tumor cells thereby giving tumors an opportunity to evade immune surveillance (Wang et al., 2017). Other studies have demonstrated that IL-17 promotes neutrophil and other immune cell recruitment to the tumor microenvironment which further aggravates pro-inflammation that in turn supports tumor growth and survival. Such a double function of IL-17 in the enhancement of both immunity and oncogenesis makes the targeting of this cytokine very complicated in treatment approaches (Karakasheva et al., 2018).

IL-17 production is mainly attributed to T helper 17 (Th17) cells; however, this cytokine has been actually shown to influence multiple facets of the tumor microenvironment. For example, Chang et al. (2014) reported that Th17 cells are very critical pathogenesis in lung cancer; logically, it should be combined with immune responses and tumoral behavior modulation by IL-17. Wakita et al. (2010) showed that IL-17-producing γδ T cells facilite tumor progression through angiogenesis, the most important process for both tumors growth and metastasis. This ability for angiogenesis underlines further the importance of IL-17 as a marker for aggressive tumor phenotypes. In another study by Zhao et al.,(2019) it was demonstrated that IL-17 induces the expression of VEGF in non-small cell lung cancer (NSCLC) via STAT3 signaling pathway signifying also here the role of IL-17 in angiogenesis which then leads to tumor growth. IL-17 can also stimulate angiogenesis, and therefore, its measurement may reflect the vascular status and general aggressiveness of the tumor. In this regard, studies have revealed that IL-17 could be used as a predictive marker in most types of cancer. For example, the levels of IL-17-producing CD8(+) T cells (Tc17 cells) were associated with both tumor progression and overall survival in gastric cancer (Yuan et al., 2012). This research indicates that measurement of IL-17 levels reflects tumor aggressiveness and patient prognosis; therefore, it is a good diagnostic tool. The other study by Harlan et al. (2011) provided an illustration for colorectal cancer about how tumors regulate immune cell profiles through cytokines like IL-17 which influences the tumor microenvironment and correlates with specific immune responses. This could be utilized to assess where an individual is with respect to disease state and progression; therefore, this further provides an argument for use in CRC diagnosis.

CONCLUSION

High levels of salivary IL-17 are linked to OSCC and its level changes with the site of the tumor lesion, which suggests IL-17 as a good easy test for finding and watching OSCC. Being male or female does not seem to affect IL-17 levels.

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