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Evaluation of salivary IL-10 and IL-8 as predictive biomarker in patient with oral and oropharyngeal squamous cell carcinoma

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Abstract

Background: The incidence and mortality rates of oral squamous cell carcinoma (OSCC) vary globally, yet the cancer continues to pose significant morbidity and mortality risks. This study sought to assess salivary IL-8 and IL-10 as biomarkers for identifying malignant oral lesions.

Methods: Between March 2022 and May 2023, a case-control study at the Dental Center of New Baquba, Diyala, Iraq, collected saliva for IL-8 and IL-10 measurement via ELISA. An independent t-test compared IL-8 and IL-10 means based on age and gender, while ROC analysis gauged specificity and sensitivity.

Results: The prevalence of OSCC was evenly distributed across genders, with a higher occurrence among patients aged over 55 years (87.5%). OSCC distribution in the oral cavity indicated the lips as the most affected area (40.0%), followed by the tongue (37.0%), and the hard and soft palate (12.50%). Statistically significant differences were noted in IL-10 and IL-8 levels between the study and control groups across all age brackets, except for IL-8 in the age ≤55 ($p \leq 0.05$). Elevated mean levels of IL-10 and IL-8 were observed in the older age group (>55), with IL-10 showing a greater increase in males (71.99±3.6), while IL-8 levels were higher in females. IL-10 demonstrated higher sensitivity (90%) and specificity (60%) with an optimal cutoff point value of 43.29, whereas IL-8 exhibited a sensitivity of 80% and a specificity of 47.5% with an optimal cutoff point value of 44.48.

Conclusion: Individuals aged over 55 showed higher IL-10 and IL-8 levels, particularly in males for IL-10 and in females for IL-8. Salivary biomarkers hold promise for early OSCC detection.

Keywords: Saliva, OSCC, IL-8, IL-10, Predictive Biomarker, Iraq

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Background

Cancers diagnosed in the head and neck region poses a considerable public health threat, with around 900,000 new cases and half million deaths worldwide in 2020. Oral squamous cell carcinoma (OSCC) is the predominant type, representing ninety percent of all oral cancer cases [1]. OSCC is a preventable condition, as it can be detected early and treated effectively, with a “five-year survival rate” covering eighty percent of patients diagnosed with early-stage (T1) tumors [2]. However, the incidence of OSCC has been increasing over the past decade, with a 50% rise reported in some regions [3]. OSCC development is influenced by various risk factors. Cigarette smoking, snuff and alcohol use are linked to more than 90% of oral cancer cases, showing a synergistic impact [4]. The oral tongue is the most common subsite affected by OSCC, accounting for up to 50% of all cases, and is associated with higher mortality rates than OSCC in other subsites, such as the floor of the mouth, gingivae, and retromolar trigone [5]. The diagnosis of OSCC at an early stage is critical to prevent extensive treatment and improve patient outcomes. Biomarkers, measurable biological indicators, offer promise for early detection and monitoring. Salivary biomarkers, like DNA, RNA, and proteins, show potential for OSCC diagnosis [6]. Interleukin-10 (IL-10) and interleukin-8 (IL-8) are potential OSCC biomarkers. IL-10, a cytokine, suppresses immune activity by inhibiting pro-inflammatory cytokines like TNF-α, TNF-γ, and IL-1. Its primary source of production is macrophages, although various cell populations, such as T-helper 2 cells, T-cytotoxic 2 cells, B cells, and keratinocytes, can also produce IL-10. IL-10 has been shown to promote tumor growth by inhibiting apoptosis and stimulating cancer cell growth [7]. Elevated IL-10 levels in saliva are a potential OSCC biomarker [8]. IL-8, a prototypical CXC chemokine, induces angiogenesis, neutrophil chemoattraction, and transendothelial migration. Its overexpression in head and neck squamous cell carcinoma (HNSCC) suggests its role in tumor growth and neovascularization [9]. Few investigations have explored IL-8

levels in OSCC patients' saliva, yet some studies propose its involvement in precancerous conditions [10,11,12]. Our research endeavors to examine salivary IL-8 and IL-10 as molecular indicators for diagnosing malignant oral lesions. By examining the efficacy of these biomarkers in diagnosing OSCC, we aspire to bridge the gap in understanding the role of IL-8 and IL-10 in oral cancer detection [13]. Furthermore, findings of this study offer valuable insights into enhancing patient prognosis and improving patient outcomes, and potentially paving the way for more effective diagnostic and therapeutic strategies in the future.

Methods

Study design and participants

A case-control prospective study was performed at specialized dental center of New Baquba, Diyala, Iraq between March 2022 and May 2023.

Inclusion and exclusion criteria

Adult patients diagnosed with oral squamous cell carcinoma (OSCC), aged 45 years or older, of both genders, were recruited. Exclusion criteria included salivary gland disorders, recent medication use, active infectious diseases, collagen vascular diseases, diabetes mellitus, hypertension, bleeding tendency, pregnant and lactating women, and unwillingness to participate in the study.

Samples Size

Analysis of data from the Iraqi Cancer Registry [14], covering cases reported between 2000 and 2008, indicated that oral cancer constituted 2.0% of all reported cancers. With a significance level of 5.0% and 95.0% power. We estimated a sample size of 31 participants, allowing a 30% dropout rate to accommodate unforeseen circumstances such as insufficient records, specimen inadequacy, and other unexpected issues. The final study sample comprised 40 participants.

Study participants

The research involved two distinct groups. Group 1 comprised 40 patients diagnosed with OSCC, classified clinically based on TNM staging (stages I to IV) and histopathologically categorized as "well," "intermediate," or "poorly differentiated" tumors. Group 2 consisted of 40 healthy volunteers serving as controls. OSCC patients were recruited from the oral and maxillofacial surgery department at Baquba Teaching Hospital, while control subjects were selected from the dental center in New Baquba, Diyala, Iraq. This stratification ensured a comprehensive comparison between OSCC patients and healthy individuals in terms of clinical and histopathological parameters.

Procedures and analysis

Prior to sample collection, participants refrained from consuming food or beverages for a minimum of one hour. Unstimulated whole saliva was gathered by instructing participants to accumulate saliva in their mouths for five minutes without swallowing, then expectorate it into a sterile container. Subsequently, saliva samples underwent centrifugation at 3000 rpm for 10 minutes using a laboratory Centrifuge Hermle Z 380. The resulting supernatants were carefully transferred to Eppendorf tubes and stored at -20°C until analysis. The concentrations of IL-8 and IL-10 in the saliva samples were quantified utilizing the quantitative sandwich ELISA technique. This analysis utilized a standard human IL-8 and IL-10 ELISA kit (Dia-clone, Besancon Cedex, France). Sociodemographic factors were analyzed descriptively. An independent t-test was recruited to compare means of IL-8 and IL-10 correlation based on age and gender. Specificity and sensitivity were determined through ROC analysis. The entire process was conducted following established protocols to ensure accuracy and consistency in the results.

Results

Table 1 present the sociodemographic factors. The prevalence of OSCC was equally distributed among the two genders, but was higher among the patients aged more than 55years (87.5%). The most of the recruited healthy volunteers were of an age less than or equal to 55(77.5%). The data in Table 2 indicates that among the diseased participants, the most frequent grade was well (42.50%) followed by Moderate (35%) and Poor (22.5%). The distribution of OSCC in the oral cavity indicated that the lips are the most affected area (40.0%), followed by the tongue (37.0%), and the hard and soft palate (12.50%). According to the TNM stages, stage 1 was the most prevalent (65.0%) among the study group. The study results showed that there was a statistically significant difference of Il-10 and Il-8 between both study and control group among all age groups except for Il-8 in the age ≤55 at $p \leq 0.05$. Higher mean levels of Il-10 and Il-8 were shown in the study group of older age > 55 with greater increase of Il-10 in males (71.99±3.6) while higher levels of Il-8 were found in females. The results showed that there was no statistically significant difference in the mean levels of Il-10 and Il-8 at different disease grades, site or TNM stage (Table 4). ROC analysis (Figure 1, Table 5) comparing both levels of Il-10 and Il-8 showed greater sensitivity (90%) and specificity (60%) for IL-10 measurement, with an optimal cutoff point value of 43.29. On the other hand, IL-8 showed a sensitivity of 80% and a specificity of 47.5%, with an optimal cutoff point value of 44.48.

Table 1: Distribution of subjects by demographic data (n=80)

Groups	Variables	Categories	N (%)
Study (n=40)	Age (years)	<=55	5 (12.5)
		> 55	35(87.5)
	Gender	Male	20(50.0)
		Female	20(50.0)
Control (n=40)	Age (years)	<=55	31(77.5)
		> 55	9(22.5)
	Gender	Male	24(60.0)
		Female	16(40.0)

Table 2: Distribution of subjects by grade, site, and TNM stage (n=80)

Variables	Categories	N (%)
Grade	Well	17 (42.5)
	Moderate	14(35.0)
	Poor	9(22.5)
Site	Lips	16(40.0)
	Tongue	15(37.5)
	Head and Soft palate	5(12.5)
	Others	4(10.0)
TNM stage	1	26(65.0)
	2	14(35.0)

Table 3: Descriptive and statistical test of Il-10 and Il-8 among groups (n=80)

Variables	**Categories**		**Groups**					
			Study		**Control**			
			Mean	±SE	Mean	±SE	T test	P value
Age	<=55	Il10	60.942	7.263	45.200	2.148	2.605	**0.014**
		IL8	54.782	7.537	45.182	1.196	1.258	0.274
	> 55	Il10	70.054	2.704	39.988	5.278	5.039	**0.000**
		IL8	55.133	2.503	40.043	5.757	2.638	**0.012**
Gender	Males	Il10	71.994	3.596	42.326	2.837	6.565	**0.000**
		IL8	51.905	2.689	42.235	2.319	2.738	**0.009**
	Females	Il10	65.836	3.564	46.580	2.792	4.254	**0.000**
		IL8	58.274	3.783	46.711	1.755	2.773	**0.010**
Total		Il10	68.915	2.547	44.027	2.039	7.628	**0.000**
		IL8	55.089	2.347	44.025	1.581	3.910	**0.000**

Table 4: Descriptive and statistical test of Il-10 and Il-8 among Grade, site and TNM stage (n=80)

Variables	Categories	IL10		IL8	
		Mean	SE	Mean	SE
Grade	Well	67.397	4.506	55.823	4.177
	Moderate	68.268	4.492	55.626	3.207
	Poor	71.175	4.080	52.868	5.160
	F		0.212		0.125
	P value		0.810		0.883
Site	LIP	62.886	3.610	58.238	3.525
	Tongue	71.812	3.730	51.325	4.335
	Head & Soft palate	66.536	9.527	51.266	6.532
	Others	85.140	6.231	61.390	4.149
	F		2.577		0.906
	P value		0.069		0.448
TNM stage	1	68.068	2.911	54.651	3.227
	2	70.489	5.004	55.903	3.153
	T test		0.449		0.251
	P value		0.656		0.803

Table 5: ROC analysis comparing sensitivity and specificity of Il-10 and Il-8 (n=80)

Area Under the Curve	Area	P value	Optimal cut off point	% Sensitivity	% Specificity
Test Result Variable(s)					
IL-10	0.872	**0.000**	43.29	90	60
Il-8	0.732	**0.000**	44.48	80	47.5

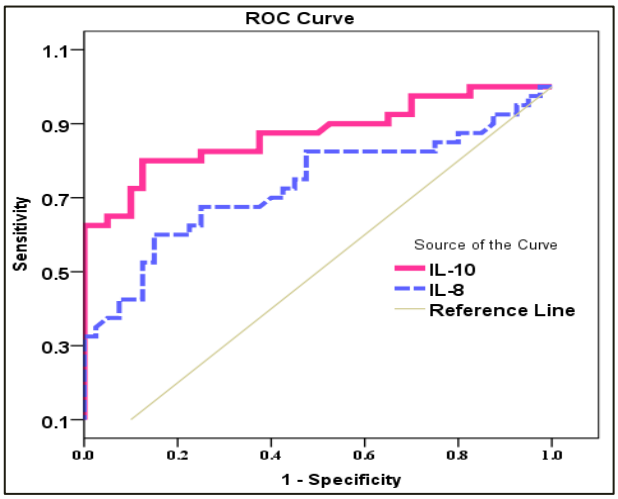

Figure 1: ROC analysis curve

Discussion

Oral squamous cell carcinoma, commonly referred to as OSCC, is a significant type of epithelial cancer that requires the attention of dental professionals. This malignancy is often detected late in its development, leading to a poor prognosis [15]. Additionally, treatment options can be both expensive and result in disfigurement. OSCC exhibits notable complexity and heterogeneity. Its pathogenesis is influenced by a variety of risk factors, such as tobacco, alcohol, viruses, and diet, among others, which can exert a carcinogenic impact on the respiratory and digestive systems' normal cells [16,17]. Oral squamous cell carcinoma has the potential to arise in any oral cavity site, although the tongue, lower lip, and mouth floor are the most commonly impacted areas. These regions are linked to a heightened risk of regional lymph node metastasis and distant organ spread [18]. Currently, the identification of OSCC is established through a thorough clinical assessment and histopathological evaluation of questionable regions. The Cancer Genome Atlas (TCGA) has reported that a significant dataset of proteomics/genomics did not enhance the prognostic potential of conventional clinical variables in various cancer patients [16]. Several ongoing investigations are currently in the exploratory stage, aiming to identify biomarkers for oral cancer. However, further validation is necessary to establish their clinical utility practice [15,16,18]. The technique of liquid biopsy is currently receiving significant attention as a means to identify the molecular characteristics of cancers in both early and late stages. Saliva is considered a highly indicative body fluid for liquid biopsy in OSCC due to its accessibility, ease of use, and close proximity to OSCC cells [19-21]. Saliva has been suggested as a valuable tool for early detection, characterization of the molecular profile of cancer patients, and the creation of a personalized treatment plan. Additionally, it enables the monitoring of response to therapy and the detection of cancer recurrence. A notable drawback in using saliva for diagnostics is that the concentration of informative analytes is often lower in saliva compared to serum, posing a challenge for accurate detection [22]. Recent advancements in highly sensitive techniques have eliminated the previous limitations of detecting lower levels of analytes in saliva. There are compelling reasons to utilize saliva as a diagnostic fluid. This diagnostic method satisfies the requirements for cost-effective, non-invasive, and user-friendly measures. Saliva presents numerous advantages over serum as a clinical tool, such as its easy collection, storage, and shipping, as well as its cost-effectiveness and the possibility of obtaining sufficient quantities for analysis. The implementation of noninvasive collection techniques for patients significantly decreases their anxiety and discomfort while also facilitating the acquisition of repeated samples for monitoring purposes. Saliva is a more convenient diagnostic tool due to its non-clotting nature, which reduces the need for extensive manipulations during procedures [23]. The cytokines are a group of chronic inflammatory mediators that have been linked to the development of cancer. The matrix proteins undergo alterations and angiogenesis is promoted in the tumour cell s. The multiplex cytokine test demonstrated efficacy in identifying and measuring cytokine concentrations in the saliva of individuals with HL PVL, at varying clinical stages of OSCC, as well as in healthy individuals. Our study has revealed the presence of two biomarkers, namely IL-10 and IL-8, which exhibit a significant difference in their expression levels between OSCC and control groups [24]. IL-8, also known as a chemokine, is generated by various blood and tissue cells and functions as a chemoattractant cytokine. IL-8 plays a role in immune surveillance, inflammation, and angiogenesis, as documented in literature [25]. According to previous research [26-28], IL-8 has been found to function as a strong angiogenic agent in tumour by regulating the proliferation and migration of endothelial cells. Prior investigations have been conducted to identify the function of IL-8 in OSCC tissue specimens, cell culture homogenates, and tissue lysates utilizing various techniques. Previous findings [29,30] indicate an elevated expression of IL-8 in HNSCC, implying its involvement in tumorigenesis. However, literature on IL-8 levels in OSCC patients' saliva is scarce, with its role in precancer relatively unexplored. Lee et al. [31] and Rajkumar et al. [32] identified IL-8 as a salivary biomarker with moderate sensitivity and specificity. ELISA emerged as the superior test for IL-8 detection in saliva. Pooling data yielded sensitivity confidence interval (CI) ranging from 0.49 to 0.90 and specificity CI ranging from 0.58 to 0.97. In 2005, Rhodus et al. [33] reported elevated salivary IL-8 levels in OSCC patients compared to those with oral preneoplastic lesions. Similarly, other studies observed increased IL-8 levels in OSCC saliva compared to controls. Lee et al. [31] noted upregulated IL-8 in early OSCC stages but found no distinction between early and advanced disease stages. Dikova et al. [34] suggested saliva-derived IL-8 as a potential discriminator between OSCC, OL patients, and healthy controls. IL-10 is implicated in tumorigenesis and metastasis by promoting tumor growth and inhibiting apoptosis. Elevated serum IL-10 levels are noted in hematopoietic and solid tumor patients [35]. Research on salivary IL-10 levels in cancer remains scarce. Hamzavi et al. [36] examined IL-10 tissue expression, serum, and salivary levels, noting no correlation among them in controls or OSCC subjects, indicating varied cytokine regulation across bodily compartments. Alhamarneh et al. [37] observed elevated serum IL-10 levels in advanced head and neck squamous cell carcinoma stages, excluding oral cavity tumors. These findings underscore the complex cytokine dynamics in cancer progression. Previous studies [38,39] indicate that elevated IL-10 expression correlates with OSCC aggressiveness and serves as an independent survival predictor, especially in early-stage patients. IL-10's role in immunosuppression and anti-tumor immunity suppression contributes to its pro-tumorigenic effects and tumor aggressiveness. Additionally, genetic polymorphisms in the IL-10 gene promoting high IL-10 expression are strongly linked to oral squamous cell carcinoma risk [37,40,41]. These findings highlight IL-10's multifaceted involvement in OSCC pathogenesis and its potential as a prognostic marker.

Conclusion

Significant differences in IL-10 and IL-8 levels were noted between the study and control groups across all age categories. OSCC cases are often identified in advanced stages. Elderly individuals (>55) displayed elevated mean IL-10 and IL-8 levels, notably in males (71.99±3.6) for

IL-10 and higher levels of IL-8 in females. Salivary biomarkers may aid in early OSCC detection, but larger sample sizes encompassing various disease stages and sites, along with prospective studies in treated patient cohorts, are essential to validate these findings...

Abbreviation

OSCC: Oral Squamous Cell Carcinoma; HNSCC: Head and neck squamous cell carcinoma; IL-8: Interleukin 8; IL-10: Interleukin 10; ELISA: Enzyme-Linked Immunosorbent Assay; SD: Standard Deviation; CI: confidence interval; TNM staging: tumor (T), node (N), and metastasis (M); TCGA: The Cancer Genome Atlas

Declaration

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Availability of data and materials

Data will be available by emailing gheny@uodiyala.edu.iq

Authors' contributions

All authors are equality conceived and designed the study, analyzed and interpreted the data; drafted the manuscript; revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical approval was obtained from the Ethics Review Committee, College of Medicine, University of Diyala, Iraq (Ref No: 19-2022). Informed consent was obtained from the participants before filling out the survey questionnaire

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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