SALIVARY IL-6 AND IL-8 AS POTENTIAL BIOMARKERS FOR ORAL SQUAMOUS CELL CARCINOMA: A LITERATURE REVIEW

Fitrah Utari Bakti[*], Riani Setiadhi[**]

*Oral Medicine Residency Program, Faculty of Dentistry Universitas Padjadjaran, Indonesia
**Oral Medicine Department, Faculty of Dentistry, Universitas Padjadjaran, Indonesia.
Corresponding author: fitrahutari@ymail.com

KEYWORDS	ABSTRACT
Cytokines, interleukin-6, interleukin-8, oscc, saliva	***Introduction:*** *Early identification of oral squamous cell carcinoma (OSCC) is a crucial factor influencing the disease's prognosis. The elevated levels of IL-6 and IL-8 in saliva are a result of their overproduction in the cancer microenvironment. The production of salivary cytokines, particularly IL-6 and IL-8, has a role in cancer development. These cytokines may also be used as biomarkers. The use of saliva as a biomarker is quite interesting because it is in direct contact with cancer cells, easy to access, non-invasive, and cost-effective.* ***Aim:*** *The aim of this literature review was to assess the potential of salivary IL-6 and IL-8 as biomarkers for OSCC early diagnosis.* ***Review:*** *The initiation of carcinogenesis in the oral cavity is actively repressed by inflammation and cell-mediated immunity. IL-6 and IL-8 are two important molecules involved in stromal-to-cancer cell communication. These cytokines play a major part in the growth, progression, metastasis, and recurrence of OSCC. Several studies have found that OSCC patients have higher levels of salivary IL-6 and IL-8 than healthy people and patients with an oral potentially malignant disorder (OPMD).* ***Conclusion:*** *Salivary IL-6 and IL-8 are very promising potential biomarkers for the early diagnosis of OSCC. Further research is still needed to validate and use these biomarkers clinically.*

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the world's major health problems with high mortality and morbidity rates.[1,2,3] An annual report lists 145,343 deaths and 300,373 new cases.[2,4] According to the World Health Organization, OSCC is the 10th most prevalent cancer in women and the 6th in men in developing countries.[3,5,6] Preventative measures, early detection, and treatment can significantly lower the mortality rate of this cancer.[3] Cancer prevention and therapeutic approaches have not improved patient prognosis.[7,8,9]

OSCC diagnosis is quite challenging, because clinically, the lesions in the early stages may resemble benign lesions or be preceded by an oral potentially malignant disorder (OPMD).[10,11,12] A late diagnosis of OSCC affects more than 50% of patients in the form of cancer with a large primary tumor that has spread to the lymph nodes or to distant locations.[4,11] Patients with early diagnoses have a higher 5-year survival rate compared to

patients with late diagnoses.[12] The 5-year survival rate of patients with OSCC who were diagnosed at stage I is 72-90%, at stage II, 39-85%, 27-70% at stage III, and at stage IV, is 12-50%.[5] Early identification of OSCC is one of the primary variables impacting the prognosis of this devastating disease.[5,6,10,11]

The "golden standard" for diagnosing OSCC is a biopsy.[5,7,10,12,13] Chemiluminescent lighting, brush biopsy, vital staining, confocal microscopy, and narrow-band imaging are all methods for forecasting the development of this cancer. This technique is not only invasive, but also expensive and impractical.[8,10,12] Many recent studies have concentrated on the examination of body fluids, which is also called "liquid biopsy."[7,11] The use of saliva is quite attractive because it has direct contact with cancer cells continuously, has easy accessibility, is non-invasive, and is cost-effective.[10] The composition of saliva can reflect cancer characteristics through various specific biomarkers. According to the studies, OSCC-related changes in saliva can be observed at the transcriptomic, genomic, proteomic, metabolic, and microbiome levels.[5,6,7] Recent proteomic studies have shown that saliva provides new information describing the relationship of salivary proteins, such as cytokines, with various pathological conditions.[5,7]

The pathophysiological control of cancer growth and progression is significantly influenced by pro-inflammatory cytokines.

These cytokines have been proposed as potential oral cancer biomarkers, whereas IL-6 and IL-8 are biomarkers in hepatocellular carcinoma and oral lichen planus (OLP). These two cytokines are not only found in tissues and serum but can also be found in saliva.[14,13] The aim of this literature review is to determine the potential of salivary IL-6 and IL-8 as biomarkers for early diagnosis in OSCC.

REVIEW

Oral Squamous Cell Carcinoma

The most typical type of head and neck cancer is squamous cell carcinoma.[15,16,17] Cancer can appear in the upper digestive tract and all parts of the respiratory tract. The oral cavity is where squamous cell carcinoma typically develops.[15] The sixth most prevalent cancer in the world is OSCC.[4,18] This cancer affects roughly 4% of all cancer cases in Western countries, whereas it affects 40% of cancer cases in India and Southeast Asia.[10]

OSCC develops from the oral mucosal epithelium. The tongue is where cancer most frequently occurs; however, cancer can also develop on the lips, floor of the mouth, hard palate, retromolar trigone, alveolar ridge, buccal mucosa, and gingiva.[17,18,19,20,21] OSCC generally occurs in elderly men, but the incidence of this cancer has increased in younger age groups in many countries.[9,17,19,20,22] Smoking, excessive alcohol use, chewing tobacco, betel nut and areca nut chewing, poor oral hygiene,

radiation exposure, exposure to chemical carcinogens, infection, and immunosuppression are risk factors for squamous cell carcinoma.[2,10,20,21,23] The pathogenesis of squamous cell carcinoma is still controversial. Several researchers agree with the theory related to the accumulation of genetic and epigenetic changes that affect protein expression and cause changes in various signaling pathways.[9,10,17]

Salivary IL-6 and IL-8 Biomarkers and Carcinogenesis

Biomarkers are molecular markers that can be useful for the diagnosis, treatment monitoring, and prognosis of certain diseases or conditions.[6] Biomarkers can be genes, proteins, enzymes, and hormones.[24] Biomarkers can be classified based on disease states, biomolecules, or other criteria.[6] Biomarker validation must pass through three levels of stages, starting with exploration and validation, until it can finally be used clinically.[24]

A variety of specific biomarkers can use the complexity of salivary composition to represent tumor features.[5,6] The secretions of the minor and major salivary glands, serum, gingival sulcus fluid, fungi, viruses, bacteria, and bacterial products, food residues, desquamated epithelial cells, and other subcellular components are all included in saliva. Saliva is composed of 95% water, proteins (cytokines, antibodies, antimicrobial compounds, and enzymes), minerals, electrolytes, nucleic acids, and hormones. Saliva is quite interesting because it provides several potential benefits, such as prognosis, early diagnosis, treatment, and post-therapy monitoring.[5] The utilization of saliva samples offers various advantages compared to serum and tissue, including: 1) saliva is in direct contact with cancer cells; 2) saliva sampling procedures are easier; 3) non-invasive procedures; 4) handling of saliva samples is safer; 5) distribution and storage of saliva samples is easier; and 6) they are cost-effective for both patients and researchers.[7,13,14,24]

Several studies have revealed that alterations produced by OSCC can be identified in saliva. Changes in the genome, transcriptome, proteome, metabolism, and microbiome can all be observed. Recent proteomic research showed that saliva provides information about the relationship of salivary proteins, such as cytokines, with various pathological conditions.[5,7] Cytokines are tiny proteins that participate in intercellular signaling, which is cell connection and communication. Cytokines help the body's immune system respond to damage, infection, inflammation, cancer, and other disorders. Cytokines can be classified based on secretion cells and their function, which are monokines, lymphokines, interleukins, and chemokines. Monokines and lymphokines are produced by monocytes and lymphocytes. Interleukins are proteins produced by T helper (Th) cells that have an influence on leukocytes. Chemokines have a

chemotactic function and play a role in leukocyte activation and migration in various immune responses.[12]

Cytokines have a role in cancer's initiation, development, invasion, and metastasis in the tumor microenvironment. Inflammation and immune responses have important roles in tumorgenesis. The inflammatory response controls intricate relationships between cancer cells and stromal cells and affects different facets of tumor growth and development. Cancer and stromal cells interact as a result of inflammation, which also regulates the development of tumors that secrete cytokines, growth factors, proangiogenic factors, and extracellular matrix remodeling enzymes.[10,12]

IL-6 is a group of interleukin cytokines, which are glycosylated proteins with a molecular mass in the range of 21–28 kDa.[12] These cytokines serve a variety of purposes, including being crucial for the development of T cells, B cells, endothelial cells, osteoclasts, neurons, and tumor angiogenesis.[14] IL-6 also has functions in activating STAT3, an oncogene and immunosuppressive marker, STAT3 then activates VEGF and supports the survival and proliferation of OSCC cells.[23] IL-6 also inhibits the tumor suppressor gene p53. IL-6 has been shown in numerous investigations to be capable of having two effects at once, i.e., stimulating the growth of certain cell types and inhibiting the growth of other cell types; therefore, these cytokines

have the ability to suppress or induce many types of tumors.[5,12]

A subset of chemokine cytokines known as CXCL8 is named IL-8. The molecular weight of the cytokine, a small soluble peptide, is somewhere between 8 and 10 kDa.[12] IL-8 exhibits chemotactic action against basophils, neutrophils, monocytes, eosinophils, dendritic cells, mast cells, natural killer (NK) cells, and lymphocytes.[5,8,14] Neutrophils and macrophages produce IL-8 in response to a variety of internal and external variables, including environmental, stress, and chemical factors. CRCX-1 and CRCX-2 receptor activity is impacted after IL-8 is activated. Additionally, IL-8 plays a crucial role in the epithelial transition, in addition to its direct implications for tumor proliferation. The mobility of vascular endothelial cells, neutrophil recruitment, proliferation, angiogenic potential, and resistance to apoptosis and metastasis are characteristics of this process.[5,8,23]

Salivary IL-6 and IL-8 as Potential Biomarkers for Oral Squamous Cell Carcinoma Early Diagnosis

The epidemiology of OSCC suggests that patients diagnosed early have a higher 5-year survival rate than those diagnosed later. The need for better early detection and tumor aggressiveness prediction techniques is highlighted by these findings.[5] Numerous studies have revealed that cell-mediated immunity and inflammation both actively

inhibit the growth of carcinogenesis in the oral cavity.[12] Some of the important chemicals involved in the interaction between stromal cells and cancer cells include pro-inflammatory cytokines, including IL-6 and IL-8.[10] The high levels of these two cytokines in the circulation and fluids surrounding the affected tissues are due to excess IL-6 and IL-8 production in the tumor microenvironment, such as saliva in cases of OSCC. IL-6 and IL-8 have critical roles in OSCC growth, progression, recurrence, and metastasis. The mechanism through which IL-6 and IL-8 influence the formation of OSCC is currently unknown. Researchers are continuously attempting to discover the answer through various investigations and hypotheses.[12]

One of the cytokines released in abundance in the tumor microenvironment is IL-6. These cytokines are secreted by cancer cells, tumor-associated macrophages, and tumor-associated fibroblast cells. The production of IL-6 through the autocrine and paracrine signaling pathways has an impact on the growth of tumors. The trans-signaling mechanisms activate pro-inflammatory pathways. IL-6 has pleiotropic properties, which are essential for maintaining tumor-host homeostasis. IL-6 modulates the inflammatory response during the acute phase by increasing the expression of anti-inflammatory molecules and reducing the expression of pro-inflammatory cytokines. Poor prognosis and lower survival rates are linked to high levels of IL-6 in the systemic circulation, whereas low levels of IL-6 signify a positive response to treatment.[12]

IL-8 contributes to the inflammatory response by attracting granulocytes, particularly neutrophils, to the infection site and promoting phagocytosis. The pro-angiogenic qualities of these cytokines contribute to the growth, progression, and spread of cancer. Numerous cancer cells, infiltrating neutrophils, tumor-associated macrophages, and endothelial cells express the IL-8 receptor. Tumor cell IL-8 secretion regulates neutrophil entry into the tumor microenvironment and secretes enzymes that promote tumor cell proliferation, development, and metastasis. Several factors, including inflammatory signals (IL-1, TNF-α), death receptors (DR5, Fas), cellular stress (anoxia, hypoxia), promote IL-8 production in cancer cells, and others. Such stimulation activates the nuclear factor-kappa-B pathway, which in turn activates the synthesis of IL-8. Through autocrine signaling pathways, IL-8 promotes cancer cell proliferation and survival. These cytokines also stimulate endothelial cell receptors, promoting the angiogenic response that leads to endothelial cell proliferation, survival, and vascular migration.[12]

Pro-inflammatory cytokines, salivary IL-6 and IL-8, have received a lot of research attention. The potential of these two cytokines as biomarkers for early detection of oral cancer is quite promising.[12] Numerous studies have found that OSCC patients have higher

levels of salivary IL-6 and IL-8 than the control group and the OPMD group (Table 1).[6,10,12] Rezaei F et al. stated that the levels of IL-6 and IL-8 in serum and saliva in the OSCC patient group were higher and statistically significant compared to the control group. IL-8 and IL-6 levels in saliva were 4.8 and 6.2 times higher than in serum, respectively. These findings show that both cytokines can be useful potential biomarkers for the early detection of OSCC. Salivary levels of both cytokines may be even more beneficial than serum levels.[14]

In contrast to healthy people, OSCC patients had statistically significant increased levels of salivary IL-6 and IL-8. According to Ferrari E et al., OPMD patients had statistically significant higher salivary IL-6 and IL-8 levels than controls but a lower level than OSCC patients. Salivary IL-6 and IL-8 levels may be used as a reference to distinguish between oral squamous cell carcinoma, OPMD, and healthy individuals. According to this justification, the two salivary cytokines can recognize OSCC development in its earliest phases.[10]

Chiamulera MMA et al. explained that salivary IL-6 and IL-8 were the two cytokines that had undergone the most research, and all research articles stated that the levels of these two cytokines in the saliva of OSCC patients were higher than controls and OMPD patients. These results indicate that salivary IL-6 and IL-8 might be further investigated to ascertain their true potential as OSCC biomarkers.[11]

According to Roi A et al., salivary IL-6 and IL-8 levels were higher in patients with OSCC and precancerous lesions than in the control group and have proven to be promising biomarkers for the identification of OSCC.[5] Nguyen TTH et al. confirmed that several studies reported salivary IL-6 and IL-8 levels as pro-inflammatory cytokines significantly increased in OSCC patients; both salivary cytokines can be used to identify OSCC and precancer lesions.[6] Lee LT et al. discovered eight biomarkers that were more abundant in OSCC patients than in the control group, including salivary IL-6 and IL-8. The findings of this study support the use of IL-6 and IL-8 salivary biomarkers as early OSCC detection biomarkers.[23]

Rhodus et al. and Brailo et al. found that smoking had no impact on salivary IL-6 and IL-8 levels in OSCC patients. Panneer Selvam's study concluded that there was a substantial relationship between stage II and stage IV OSCC and salivary IL-6 levels. Dineshkumar et al. reported that the diagnostic sensitivity of salivary IL-6 levels was 99%, whereas Hamed et al. showed that the diagnostic sensitivity of salivary IL-6 and IL-8 levels was 80% and 100%, respectively.[14]

The most widely used immune-based tests in research to evaluate saliva samples are bead-based suspension arrays and enzyme-linked immunosorbent assays (ELISA).[5] Several important aspects must be considered regarding saliva samples and their utilization,

including the collection methods, time of collection, and type of saliva (unstimulated or stimulated saliva). Saliva sampling should be standardized due to many factors that can affect its composition, such as age, gender, diet, and oral hygiene. The sampling should be done between 8 and 10 a.m., 12 hours after the last meal, because circadian variation also affects salivary secretion. The type of saliva used for a more accurate diagnosis of OSCC is whole stimulated saliva because the concentration of analytes is more representative. The sampling analysis time should be under 5 minutes to obtain the correct biomarker quantification so that the degradation process does not occur.[5,6] Saliva samples can be kept at room temperature for 30-90 minutes before being analyzed. Thomadaki et al. suggested that saliva samples be frozen at temperatures below -20ºC immediately after collection to slow down salivary proteome degradation. Salivary samples can also be kept at -80°C for a number of years with little to no deterioration.[6] Increased salivary interleukin levels can be influenced by several factors, including dental infections, periodontal tissue infections, OLP, and others.[10]

Currently, salivary IL-6 and IL-8 have not been used in clinical practice as potential biomarkers of OSCC. Further research is needed with larger and multicentric samples to obtain standard protocols for using these biomarkers, such as saliva collection methods, saliva storage, saliva processing, and cytokine threshold values.[14,6,10,11,12] These advancements are likely to deliver significant

value, allowing authorities to design public dental health policies.[12] The application of liquid saliva biopsy as an alternative examination has great potential for the diagnosis and management of OSCC. This is expected to improve the prognosis, therapy, and follow-up of cancer management.[10]

CONCLUSION

There are increased levels of salivary IL-6 and IL-8 in OSCC patients compared to healthy individuals and OPMD patients, suggesting that these cytokines could be explored as very promising potential biomarkers for early diagnosis of oral squamous cell carcinoma in the future. Further research is still needed for these biomarkers to be validated and used clinically.

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