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Review Article

Diagnostic Strategies for Early Detection of Oral Squamous Cell Carcinoma: A Review Article

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer with high morbidity and mortality rates. Late diagnosis and high incidence rate of OSCC have become global healthcare issues. The purpose of this review was to provide an overview of the diagnostic and prognostic biomarkers in OSCC.

Methods: A literature search in PubMed, ScienceDirect, and Google Scholar was performed. The keywords "early detection", "oral cancer", and "oral squamous cell carcinoma" were searched in the title and abstract of the articles published in English from 2000 to mid-2021. The full texts of 250 articles were retrieved and only 63 articles met the inclusion criteria.

Results: In summary, all selected papers discussed the importance of early detection along with different factors and techniques to detect oral cancer. The biomarkers were divided into three groups as follows: salivary biomarkers, circulating biomarkers, and tissue biomarkers.

Conclusions: In this review article, salivary biomarkers along with the circulating and tissue biomarkers were reviewed. Besides, some detection techniques were explained.

Keywords: Mouth, Neoplasm, Precancerous

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Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and one of the main causes of cancer-related death around the world. Besides, oral cancer causes facial disfigurement and morbidity (1). The risk factors include smoking and alcohol consumption, chronic inflammation, UV radiation (lip cancer), HPV and Candida infections, immunosuppression, and genetic predisposition. Besides, oral microbiome and inflammatory cells play essential roles in the malignant transformation of the oral mucosa (2). Oral cancer patients are still diagnosed at advanced stages. Lymph node metastasis (especially to cervical lymph nodes) is an important prognostic factor and a leading cause for cancer-related death globally (3). Early diagnosis is an attractive strategy to improve survival rates in patients. Cancer control strategies depend on early diagnosis and detection which rely on clinical assessment. The delay in diagnosis can be attributed to either the patient or clinician (4). Total delay is a period of time from the patient's first awareness of symptoms to the onset of treatment. In addition to patient's awareness, clinical presentations can aid the clinician (5). Delayed diagnosis has mostly been indicated in the cases of gingival and buccal cancers (1). Screening programs such as mammography and Pap smear test are widely used for screening for breast cancer and cervical cancer; however, oral cancer is diagnosed only after progression of the disease. Therefore, conventional visual examination of the lesion under white light illumination and palpation play pivotal roles in the diagnosis of highly susceptible lesions. Based on current knowledge, tissue biopsy and histological examinations are the gold standards for accurate diagnosis of oral dysplasia at an early stage of disease progression. However, non-invasive biomarkers are required (6). According to the World Health Organization (WHO), lesions and conditions in the oral cavity, which may undergo malignant transformation, are referred to as oral potentially malignant disorders (OPMDs). It is proposed that 50% of oral cancers develop from precursor lesions. Hence, the early detection and proper management of precancerous lesions have great impacts on oral cancer prevention (7).

Methods

Search Study

A literature search in PubMed, ScienceDirect, and Google

Scholar was performed. The keywords "early detection", "oral cancer", and "oral squamous cell carcinoma" were searched in the title and abstract of the articles published in English from 2000 to mid-2021. The full texts of 250 articles were retrieved and only 63 articles met the inclusion criteria. The inclusion criteria were the early detection, biomarkers, and detection techniques.

Results

Each article was carefully read and analyzed. In summary, the selected papers discussed the importance of early detection and different factors and techniques to detect oral cancer. The biomarkers were divided into three groups as follows: salivary biomarkers, circulating biomarkers, and tissue biomarkers. Besides, some detection techniques are addressed below.

Oral Potentially Malignant Disorders (OPMDs)

OPPDs include leukoplakia, erythroplakia, erythroleukoplakia, proliferative verrucous leukoplakia, palatal reverse smoking, lichen planus, and submucous fibrosis (8,9). It is estimated that the global prevalence of OPMDs is 4.47%. However, the prevalence may vary between populations and genders. For example, it is higher in Asians and males (10). Tissue biopsy is the gold standard for cancer diagnosis. This technique is used for histological analysis and detection of early dysplastic changes. The degree of dysplastic changes in the epithelium is the main indicator of the risk of malignant transformation in OPMDs (11). Dysplasia is characterized by epithelial architectural disturbance and cellular atypia. Dysplasia is classified as mild, moderate, and severe according to the severity of epithelial involvement (12). Although histologic examination has been considered as the gold standard for the diagnosis of oral dysplastic and neoplastic changes, some other invasive or non-invasive techniques have been suggested as diagnostic tools.

Salivary Biomarkers as Non-invasive Diagnostic Tools

Saliva analysis is a noninvasive and inexpensive tool for cancer diagnosis. Compared to blood or tissue samples, saliva has a few advantages including the ease of collection, transport, and processing (13). Additionally, biomarkers in saliva are diluted and easily available. Importantly, saliva has a direct contact with oral cancer lesions, and hence is a suitable method for detecting oral cancer (14). Saliva is a biofluid that contains several proteins and factors, circulating and tissue derived cells, extracellular vesicles (EVs), DNA and RNA molecules, and different cytokines. A variety of salivary biomarkers can be detected in patients with OSCC such as cell-free DNAs (cfDNAs), circulating tumor DNA (ctDNA), EVs, and miRNAs. Necrotic and apoptotic cells can release DNA/RNA molecules into body fluids during physiologic and pathologic conditions (15). In physiological conditions, these molecules are phagocytic targets; however, they accumulate in the tissue microenvironment and biological fluids in cancers. It is believed that the increased number of apoptotic/necrotic cells in cancer patients is the underlying mechanism (15).

cfDNAs are short (70–200 bp) or long (up to 21 kb) fragments of double-stranded DNA. They can be detected in blood, saliva, plasma, urine, cerebrospinal fluid, and other bodily fluids. cfDNA is freely circulating DNA, but it does not necessarily originate from a tumor as it can be released from normal dying cells. Apoptotic tissue and hematological cells which release the DNA into the circulation are the major sources of cfDNA in a body fluid. In healthy individuals, cfDNA is found at low levels because apoptotic cells and cfDNA are cleared very quickly. However, in chronic inflammation and cancer cases, clearance is inadequate; therefore, cfDNA can accumulate. In cancer patients, cfDNAs are found in tissue samples and reflect the genetic and epigenetic alterations (16). Circulating tumor DNAs (ctDNAs) are tumor-derived fragmented DNAs which are not associated with cancer cells. Several cancer characteristics such as cellular turnover, vascularity, and drug responses are related to ctDNA concentrations. Although ctDNA is mainly released into the bloodstream, it can be found in other body fluids including saliva. Interestingly, due to less dilution and contamination, the analysis of ctDNA in saliva is highly sensitive (16).

miRNAs, small endogenous single-strand RNA molecules, are dysregulated in many diseases (17). miRNAs can control different events in cancers such as proliferation, differentiation, apoptosis, survival, motility, invasion, and metastasis (18). Salivary miRNAs are the potential biomarkers for detection of oral cancer (19). For instance, down-regulation of salivary miR-139-5p has been detected in early tongue cancer (20). In a previously published study, salivary miR-31 level was evaluated in patients with OSCC and patients with oral verrucous leukoplakia. According to the results of this study, miR-31 level was significantly increased in patients with oral carcinoma at all clinical stages, compared to that in patients with oral verrucous leukoplakia (21). A study carried out on saliva samples showed an elevated miR-21 expression level in the salivary samples of OLP, dysplastic OLP, and OSCC patients compared to those of control individuals. In addition, a significantly increased expression level of miR-31 was found in samples from dysplastic OLP and OSCC patients compared to those from healthy controls (22).

EVs are lipid bilayer-delimited particles that are naturally released from cells. EVs represent one of the intercellular communications found in tumor microenvironment (TME) and saliva. In saliva, cell-derived EVs contain some factors such as DNAs, RNAs, miRNAs, and proteins. Recent investigations have indicated that EVs may have essential roles in oral cancer growth. The most studied vesicles in tumor growth are micro-vesicles and exosomes (15). Exosomes are small membrane vesicles, ranging from 40–150 nm, which are present in the TME. Exosomes contain proteins, lipids, mRNAs, miRNAs, and mitochondrial

DNA. They are released from a variety of cells into biological fluids such as saliva, urine, semen, amniotic fluid, cerebrospinal fluid, lymph, tears, and blood in physiologic and pathologic conditions. Salivary exosomal miRNAs are considered as diagnostic biomarkers for various malignancies, including OSCC (23). For example, salivary exosomal miR-24-3p has been demonstrated as a potential biomarker for OSCC (24). In addition, tumor-derived exosomes in saliva have divergent morphological and molecular characteristics. Therefore, they can be used in the early detection of precancerous lesions and malignant transformation (15). Exosomes also play essential roles in pro-metastatic niche formation, as well as bone marrow and lymph node metastases. Exosomes can be used as a predictive tool in cancer patients and to differentiate healthy individuals from cancer patients (14). It is suggested that exosomes contribute to tumorigenesis, invasion, and metastasis (25). Besides, salivary exosomes contain elevated IgA level which has a significant role in the local immune response of the oral cavity (26). Dysregulation of some other factors and cytokines can be found in saliva. For example, a published paper revealed increased levels of salivary tumor necrosis factor alpha (TNF-α), a cell signaling protein, in patients with OSCC compared to healthy control subjects and patients with leukoplakia. The authors suggested the salivary TNF-α level as a tool for monitoring the malignant transformation of leukoplakia to OSCC (27). An early investigation on salivary and serum IL-6 levels showed significant differences in oral premalignant lesions and oral cancer. IL-6 promotes cancer cell proliferation and involves in the inactivation of the p53 tumor suppressor gene (28). Some data have shown significant correlations between salivary and serum biomarkers. However, some others have shown that biomarkers may differ between blood and saliva. For instance, extracellular RNA biomarkers in the blood are different from those in the saliva. Standard techniques for saliva collection and processing criteria play essential roles in the reliability of the analysis (29).

Circulating Biomarkers

cfDNAs are released into the bloodstream. However, a detailed study which analyzed the plasma level of cfDNAs in 390 patients (90 potentially malignant lesions, 150 OSCCs, and 150 post-treatment OSCCs) and 150 healthy controls did not find any significant difference between the examined groups. The authors proposed that due to the rich lymphatic drainage of the oral mucosa, cfDNA does not enter the bloodstream (30).

Circulating DNA (ctDNA) can be detected in different forms such as free DNA, protein-bound complexes either as free circulating molecules or encapsulated in vesicles (apoptotic bodies, microvesicles, and exosomes). It is now clear that ctDNA has an active role in carcinogenesis. CtDNA has a short half-life of around 10 to 15 minutes. It is rapidly degraded by blood nucleases and eliminated by the liver, spleen, and kidneys (31).

Circulating miRNAs or cell-free miRNAs, a class of short non-coding RNAs, are also considered as noninvasive cancer biomarkers. Interestingly, cell-free miRNAs are encapsulated in lipoprotein complexes, and hence are protected from endogenous RNase activity in body fluids (32). Circulating miRNA levels have been studied in patients with oral premalignant lesions and OSCC. For instance, the expression level of miR-21 was assessed in the serum of patients with oral submucous fibrosis (OSF) and OSCC. According to the results of this study, a significant difference was found between OSF and OSCC patients in terms of miR-21 expression level. Additionally, there was a significant relationship between the expression of miR-21 and the clinical stages of OSCC patients (33). Data collected from a previous study has shown that up-regulation of plasma miR-10b can be used as an early detection marker for oral cancer (34). In a published study, circulating miR-196a and miR-196b were assessed in plasma samples of 53 healthy individuals, 16 pre-cancer patients, and 90 oral cancer patients. The results showed a significant distinction between normal and precancerous patients and between normal and cancer patients. The authors suggested that the combination of miR-196a and miR-196b may be a useful tool for the early detection of oral cancer (35). Circulating expression levels of 3 miRNAs (miR-222-3p, miR-423-5p, and miR-150-5p) were indicated in patients with oral leukoplakia and OSCC in a published article. According to the findings of this study, miR-222-3p expression level was significantly down-regulated in leukoplakia patients compared to the normal and OSCC patients whereas miR-423-5p and miR150-5p expression levels were elevated in OSCC patients compared to the healthy individuals and leukoplakic patients. The authors suggested miR-222-3p, miR-423-5p, and miR-150-5p as potential biomarkers for the early diagnosis of OSCC and oral leukoplakia (36).

A number of scientists have indicated that circulating tumor cells (CTCs) can also be used to diagnose cancer at an early stage. CTCs are rare epithelial cells shed from primary tumor into the vasculature. CTCs are found in 30%–40% of cancer patients. CTCs can be considered as prognostic markers (37). Morphologically, CTCs are similar to the primary solid tumor cells and play crucial roles in metastasis. Hence, it is important to identify patients with CTCs to predict metastasis at an early stage. CTCs have been detected in patients with head and neck SCC and correlate with lymph node metastasis (37). CTCs undergo epithelial-mesenchymal transition (EMT), a crucial event in cancer development, to migrate to distant organs (38,39). In a previous study on OSCC patients, CTCs were detected in 12.5% of patients with OSCC. Surprisingly, significant correlations were found between CTCs and tumor size (40). Circulating tumor microemboli (CTM) are clusters of tumor cells. It is proposed that CTM can be easily caught in narrow vessels than CTCs, which provide a new suitable environment for the survival of tumor cells (25,41).

Circulating cytokines are also involved in malignant

transformation and tumor growth. The role of cytokines has been evaluated in oral premalignant lesions. According to the results of a study, cytokines such as TNF-a, TGF-β1, and IL-6 have significant effects on the risk of development of precancerous lesions (42).

Tissue Biomarkers

Aberrant levels of miRNAs have been detected in oral samples. For instance, the expression of miR-375 was significantly decreased after progression from premalignant lesion to OSCC. It is suggested that miR-375 expression level is a useful tool to identify progressive premalignant lesions from non-progressive samples (43). Recent studies have shown that OSCC parental cells release exosomes with oncogenic markers which are able to influence the surrounding TME. In OSCC, exosomes are present in TME and can increase the expression of TGF-β, a key player signaling pathway in tumor progression (44). TME consists of different cell types. Among them, cancer-associated fibroblasts (CAFs) have the capacity to transport miRNAs and proteins to cells by exosomes (45). CAF-derived exosomes enhance OSCC metastasis (46,47).

In a previous study on oral leukoplakia and OSCC samples, the expression level of p53 and epithelial growth factor receptor (EGFR) increased as the lesion progressed from non-dysplastic lesions to moderate dysplastic lesions. The authors proposed that p53 and EGFR play critical roles in the progression of premalignant lesions to carcinomas (48). Cytokeratins (CKs) are useful biomarkers for the assessment of histopathological progression of oral cancer. In a previously published paper, the expression of CK10-ab1 was assessed in keratinized squamous stratified epithelium. Sever expression of CK10-ab1 was indicated in the suprabasal layers of all specimens in normal and hyperplastic samples; however, CK10-ab1 disappeared gradually with the progression of malignant changes. Therefore, the expression of CK10-ab1 was mild in all poorly differentiated SCCs. The authors suggested CK10-ab1 as a predictable marker for the early detection of OSCC (49). A detailed investigation on CDK1 has demonstrated a higher expression level of CDK1 in OSCC samples. In this study, there was a significant correlation between the expression level of CDK1 and histological grade of OSCC. Hence, overexpression of CDK1 was found in high grade tumors (50). Moreover, cytokines and growth factors produced by inflammatory cells can be stained in tissue specimens. Increased expression levels of IL-1α, Il-1β, TGF-β, platelet-derived growth factor, and basic fibroblast growth factor have been found in both epithelium and underlying connective tissue of OSF samples (51). Besides, the expression of NF-κB has been detected in oral premalignant and malignant lesions. A previous study has reported a statistically significant gradual increase of NF-κB cytoplasmic immunostaining score from normal mucosa to OSCC (52). EMT markers can also be detected in oral potentially malignant disorders (OPMDs) s and cancer tissue samples. Expression levels of E-cadherin and vimentin, the most important EMT markers, were assessed in 64 OMPD tissue samples and 23 malignant cases of oral cavity using immunohistochemical (IHC). The results of this study showed a significantly reduced expression level of E-Cadherin in invasive carcinoma samples compared to dysplastic and carcinoma in situ cases. In this study, the expression level of vimentin was positively correlated with tumor progression (53). Additionally, early detection of OSCC can rely on the identification of new biomarkers of extracellular matrix such as matrix metalloproteinases (MMPs) (54). Higher expression levels of MMP-1 and MMP-9 have been demonstrated in OMPDs which progressed to cancer (55). Figure 1 represents a summary of the biomarkers which can be used for the early detection of OSCC.

Detection Techniques

As the early diagnosis of oral cancer is a key factor in improving the survival and quality of life, it is important to employ different detection techniques. In addition to tissue biopsy, some other helpful techniques are addressed below.

(A) Brush biopsy obtains specimens from all three layers; the basal, intermediate, and superficial layers. A brush biopsy is a non-invasive and painless technique that facilitates cytological analysis. However, the problem is that the diagnosis needs to be confirmed by tissue biopsy; therefore, it delays the diagnosis.

(B) The fluorescence method (chemiluminescence) is also used for the early detection of precancerous and cancerous lesions. It is the direct visualization of oral cavity and has a good sensitivity to detect oral precancerous lesions, but it only detects leukoplakia not erythroplakia. In addition, this technique costs quite high (6,56).

(C) Toluidine blue stains the cells with an increased amount of DNA and broader intercellular canals. A previously published study has indicated that the sensitivity and specificity of toluidine blue test are 92.6% and 67.9%, respectively, and the overall diagnostic accuracy is 80%. The toluidine blue staining is simple, rapid, and noninvasive. In addition, the application of toluidine blue can reduce the number of biopsies (57).

(D) The methylene blue staining shows the increased amount of DNA in potentially malignant cells. Accumulating data indicates that methylene blue has high sensitivity but low specificity (58).

(E) IHC uses antibodies to detect the location of proteins and other antigens in tissue samples. It is also easy to determine microvessel density by IHC (59). Occult nodal metastasis may be unnoticed in the routine pathological examination but serial sectioning and immunohistochemistry with pan-cytokeratin markers can help in the early detection of micro-metastasis (59).

(F) Liquid biopsy is a non-invasive method to detect OSCC. In contrast to tissue biopsy, liquid biopsy is easy, less invasive, and more comfortable for patients. Liquid biopsy is also a helpful method for detecting HPV DNA.

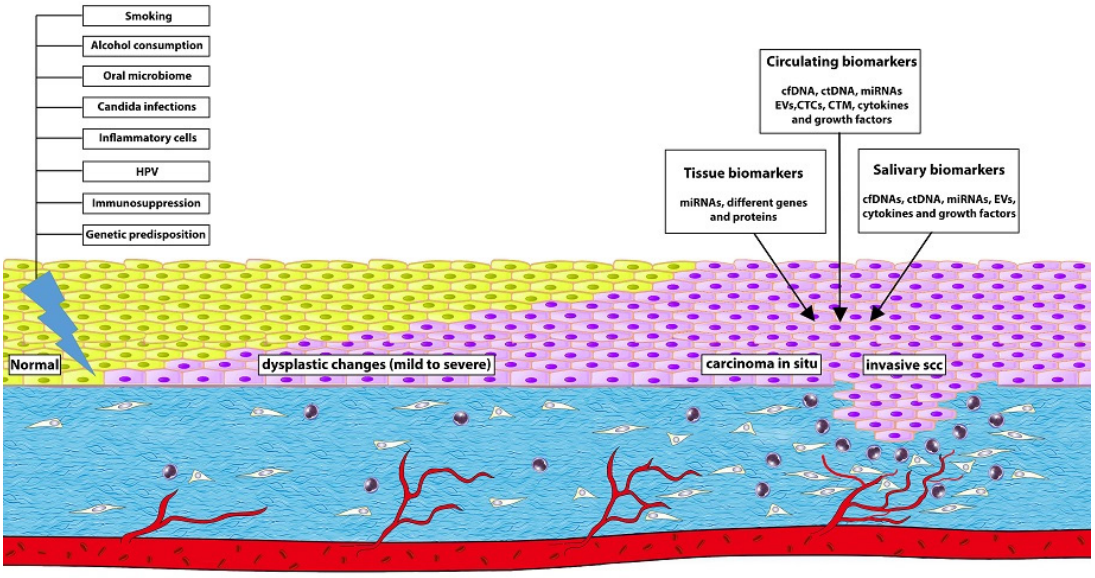

Figure 1. Overview of the major risk factors which promote oral dysplasia and develop oral cancer. Molecular biomarkers can aid in the early detection, monitoring and prognosis of oral cancer.

A previously published study has found a higher level of HPV-16/18 DNA (cfDNA) in the plasma of 14% of patients (60).

(G) Proteomics, the large-scale study of proteins, investigates different proteins expressed in cells and tissues. A variety of tools can be used for proteomic analysis such as ELISA, PCR, Western Blot, and IHC (61).

(H) For a successful result, some other methods are also helpful. For example, Sanger sequencing, q-PCR-based methods, fluorescent assays, and chromatographic methods are powerful tools for cfDNA analysis (30). miRNAs can be detected by microarray analysis, RT-qPCR, northern blotting, and in situ hybridization. Recently, nanomaterials such as gold nanoparticles, magnetic nanoparticles, silver nanoclusters, and quantum dots have been applied for miRNA detection (62). CTCs can be detected by telomerase activity, aptamer technology, and the CELLSEARCH system (41). A variety of techniques based on microfluidics such as Lab-on-a-chip, microfluidic digital PCR, microfluidic single-cell RT-PCR, digital microfluidics, and microfluidic co-culture technique based on 3D spheroids are useful in analyzing genetic mutations, TME, cell proliferation, cancer growth, and interactions between cancer cells and mesenchymal cells (61). CTCs can be detected and isolated by telomerase activity, aptamer technology, the CELLSEARCH system, and microfluidic technologies (41,61).

Therapy

After the correct diagnosis of OPMDs, a regular follow-up by an oral health professional is recommended. All risk factors such as smoking and alcohol consumption should be controlled. High-risk lesions such as leukoplakias, erythroplakias, and erythroleukoplakias with moderate or severe dysplasia should be excised. Patient follow-up at appropriate intervals is highly recommended (10). Surgical treatments such as excision, cryosurgery, and carbon dioxide (CO2) laser ablation can be useful. Accumulating evidence shows that the surgically treated precancerous lesions cannot reduce the rate of malignant transformation. It is believed that field cancerization is the underlying mechanism (8). However, premalignant lesions can be managed only by observation and administration of chemopreventive compounds including retinoids, cyclooxygenase-2 inhibitors, epidermal growth factor receptor inhibitors/antagonists, and p53 modulators. The most extensively studied chemopreventive compounds are the retinoids, but they are limited to the treatment of oral leukoplakia (63). The awareness of public and clinicians of the risk factors and early signs of precancerous lesions plays a critical role in prevention. Preventative health care strategies are important to minimize the rate of morbidity and mortality from oral cancer (63). Prevention, screening techniques, and early detection can minimize morbidity and mortality rates. Experience and training of oral health practitioners are the best diagnostic techniques (63).

Conclusion

OPMDs are considered risk factors for the development of OSCC. Avoiding smoking and alcohol consumption can have beneficial effects on oral health. It is important to detect primary OSCC at an early clinical stage. It is needed to improve the dentists' knowledge about the early detection of potentially malignant and oral cancer lesions by encouraging them to participate in cancer prevention programs. Such training programs could reduce delay in diagnosis. Besides, oral cancer awareness among the public should also be improved. In addition to clinical examination and histologic evaluation, some new techniques in the field of molecular and cellular biology are available to detect malignant transformation. When a persistent lesion is detected, a biopsy should be performed. After complete excision of the lesion, adequate follow-up and monitoring are required.

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