

Diagnosing oral squamous cell carcinoma using salivary biomarkers

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Article Info

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Abstract

Oral cancer is becoming a frightful public health issue because of its rising incidence as well as mortality rates worldwide. Out of all types of oral cancer, the oral squamous cell carcinoma is the most common malignant tumor with an incidence of about 90%. This fatal disease is diagnosed through a comprehensive clinical examination followed by the histological assessments forming the diagnostic gold standard. Although the oral cavity is simply accessible, but maximum oral cancers are usually diagnosed at the late stage. Consequently, it is necessary to implicate newer screening and early diagnosing approaches which will diminish the morbidity as well as mortality related to this disease. Saliva which is a complex biological fluid has a direct relationship with the oral cancer lesion and contains abnormal DNA, RNA, protein molecules released by the malignant cells. These can be labeled as neoplastic biomarkers proposed to play an important role in diagnostic, therapeutic and prognostic purposes for oral cancers as well as other diseases. The aim of this review paper is to concisely discuss the different types of potential salivary biomarkers as well as their interaction for the screening of oral cancers.

Introduction

Oral cancer means cancer that occurs in the oral cavity which is a debilitating disease and may affect this area during any time of the life. Out of all types of oral cancer, the oral squamous cell carcinoma is highly impacted malignant tumor which is accounted for about 70 to 90%.¹ In the developing countries, oral cancer is the most common cancer, has taken 6th position for the males following lungs, prostate, colorectal, stomach and bladder cancer as well as 10th position for the females following breast, colorectal, lungs, stomach, uterus, cervix, ovaries, bladder and liver.²

Persistence exposure to the tobacco and tobacco-like products, chewing betel quid, sustained consuming alcohol, chronic inflammation,³ and viral infections (especially oncogenic human papilloma virus) enhance the chance of occurrence of the oral squamous cell carcinoma by changing the genetic manifestation.^{3,6} Buccal mucosa, lip, alveolar ridge, retromolar trigone, hard palate, floor of the mouth, the ventral two-thirds of the tongue and oropharynx are the common oral structures prone to oral squamous cell carcinoma.⁷ The ratio of occurrence between the men and female is 1.5:1 which is mostly due to the frequency of exposure to the causative agents.⁸ As of late, the number of young generation is affecting by misusing tobacco.⁹

Saliva is a complex oral fluid that performs several physiologic functions like swallowing and tasting of foods, oral digestion, tissue lubrication, maintain tooth's integrity, antibacterial and antiviral activities.¹⁰ A different types of enzymes, hormones, antibodies, antimicrobial constituents and cytokines are available in the saliva,^{11,12} and most of them come to the saliva from blood using the cellular transportation like active transport, transcellular, passive intracellular diffusion and by extracellular ultrafiltration from the salivary glands using paracellular routes or through gingival sulcus.¹¹⁻¹⁵ Blood and saliva contain compounds which can give information about the different body's physiologic conditions (like emotional, endocrinal, nutritional and metabolic variations) as well as assist monitoring of oral and systemic health as well.

This review will discuss the current situation of oral cancer, justification of uses of salivary biomarkers and its role in the identification of oral squamous cell carcinoma.

Current Situation of Oral Cancer

Usually, development of oral cancer is strongly associated with the precancerous lesion (such as erythroplakia, leukoplakia and oral submucous fibrosis) as well as causative risk factors (like consumption of tobacco and alcohol,



infections etc.). The transformation rate of pre-cancerous lesion to cancer is about 0 to 20% within 1-30 years depending on the variety of lesions and follows a sequence of histopathological levels.¹⁶

Although having advancement in surgery, radiation therapy and chemotherapy, there is no remarkable variation in the 5 years-survival rate of oral cancer. Last several years, the survival rate is about 50% worldwide with the reason for late diagnosis, poor response of tumor to chemotherapy and radiation therapy; as well as insufficient biomarkers for early diagnosis and post-therapeutic monitoring.^{17, 18} Diagnosis of oral cancer in early stage (Stage I) has shown a mortality rate of about 20%,¹⁹ whereas late (Stage III) diagnosis causes 80% mortality.²⁰ In addition, the recurrence rate of oral cancer is very high where luckily survived patient from the first time attack has risk increased up to 20-fold for having a recurrence of this disease,^{21, 22} and is indicating the objective of early diagnosis of recurrence neoplasms.

Histological examination of recurrence cancer usually needs time to get a result. In case of imaging examination, it has no effective role in cancer screening. So, early diagnostic tools should have to implicate for getting acceptable effects on assessing the prognostic value of cancer.

Current management protocol for oral cancer consists of comprehensive clinical examination followed by histological examination which is the current gold standard for its diagnosis. Nevertheless histological assessment is in some extent subjectively and somehow not practicable because of the nature of tumor and therapeutic response of tumor.²³ So, researchers are finding a new technique which is necessary for early diagnosis as well as to increase the survival rate of this disease.

The overall situation is emphasized to increase erudition regarding biological nature of this aggressive disease to discover definitive biological indicators which will be highly effective for the screening of high-risk patients of oral squamous cell carcinoma.²⁴ The erudition of molecular pathways of this multifactorial disease is momentous for observing the genetic alterations and identify the particular biomarkers relation with growth,⁴ progression and recurrence of the tumor for early diagnostics, prognostics and treatment of oral squamous cell carcinoma using human genome database technology.²⁵ Blood and saliva are the most acceptable body fluid for recognizing the most of the biomarkers detection medium for oral squamous cell carcinoma in comparison to tissue biopsy which is invasive as well as contains plenty of biomarkers.²⁶

Saliva acts as an image of human health status

Saliva is a complex and dynamic biological fluid

that contains different types of analytes (protein, mRNA, and DNA) which serve as biomarkers for conveying message as well as clinical implications.¹⁶ The major ingredient of saliva is water approximately 99% and remaining percentage belongs to organic as well as inorganic components; in where the inorganic components mostly consist of weak and strong ions including Na⁺, Ca²⁺, Cl⁻, (HPO₂)₃, Mg²⁺, HCO₃ and NH₃. The organic components composed of body secretion products like putrefaction products; lipids, and more than 400 types of protein out of which glandular origin proteins (alpha-amylase, cystatin, histatin, lactoferrin, mucin, lysozymes, and proline-rich proteins), plasma derivatives (secretory immunoglobulin A, albumin) and transferrin are very important.²⁷

Oral cancer is one of the malignancies where examination of saliva can be the most beneficial for diagnosis due to its close relation with oral cancer lesions. Locally expressed proteins which are available in saliva [such as matrix metalloproteinase (MMP), interleukin (IL)], make saliva to be the investigation tool of choice as potential biomarkers for oral cancer.¹⁶ Saliva as an investigation tool shows merits over serum like it is non-invasive, inexpensive and easily accessible media for diagnosing, prediction of prognosis and oral cancer patient's post-therapy status monitoring.⁸ Saliva is called as "image of the health status" due to its wide range of application and raising reliability as a diagnostic tool. Researchers are highlighting their achievements to build-up its clinical benefits.

Neoplastic Markers

Biomarkers or biological markers are the measurable tools of certain biological/pathological state or detectable tools for the availability of living organism inside the body respectively. Different types of analytes or medium (like blood and saliva) usually used to analyze the biomarkers.

Substances that may be secreted from the cancer cell or from the body because of oncological reaction are referred to neoplastic markers.²⁸ Neoplastic markers are the specific, novel, or structurally altered cellular macromolecules or temporarily, spatially, or quantitatively altered normal molecules that are related with the malignant (sometimes with benign) neoplastic cells.²⁹ Types of neoplastic biomarker are shown in Table I.

Source of neoplastic markers

The collection of neoplastic biomarkers may be achieved from the following sources:^{33, 34}

Tissue- like solid tumors, lymph nodes, bone marrow or circulating tumor cells in the blood. It is important for the diagnostic pathologist.

Table I

Types of neoplastic biomarkers		
On the basis of tissue origin ³⁰		
<i>Epithelial biomarkers</i>	<i>Connective tissue biomarkers</i>	<i>Salivary gland biomarkers</i>
Cell surface biomarkers- histo-compatibility	Intermediate filament proteins- desmin	Epithelial biomarkers- cytokeratins
Intracellular biomarkers- cy-tokeratins	Other filament proteins- laminin	Myoepithelial cell biomarkers- actin, myosin
Basement membrane biomarkers - type IV collagen	Cellular enzymes - amylase, lysozyme	Serum acinar cell biomarkers- salivary amylase
Matrix biomarkers- tenascin	Cytoplasmic non-filamentous non-enzymatic proteins- myoglobin, S100 protein	Myoepithelial cells + acinar cells- S100 protein
Membrane antigen- blood group antigens such as ABH, Lewis etc	Membrane antigen- leukocyte specific antigen	
On the basis of functional utility (according to NCCN-National Comprehensive Cancer Network) ³¹		
Diagnostic biomarkers		
Prognostic biomarkers		
Predictive biomarkers {such as human epidermal growth factor receptor 2(HER2)}		
Companion diagnostic biomarkers - it may be diagnostic, prognostic or predictive, but it will use to identify a subgroup of patients with good post therapeutic response		
On the basis of biomolecules ³²		
DNA biomarkers		
RNA biomarkers		
Protein biomarkers		

Body fluids- like blood, saliva or serum (serological neoplastic biomarkers). Serological neoplastic biomarkers are usually helpful for the clinician.

Ideal neoplastic biomarker

Actually, types and uses of biomarkers have strong relationships with its properties. The usual characteristics of ideal biomarker are as follows:³⁴ a) It should be a product of neoplastic tissue which will be detectable at the initial stage of disease; b) It should be available for a particular disease in remarkable level as well as in every patient; c) Expression or out breaking to organ site should be in a definitive manner; d) Assessment of availability in the body fluid should be achieved by either non-surgically or simply approachable tissue; e) It should be proportionally related to tumor size, biological behavior, or disease progression; f) It should have relatively short half-life as well as showing temporal changes in tumor burden and making response to therapy; g) It should be presented a standardized, reproducible; and reliable objective and quantitative assay.

Justification of uses of salivary neoplastic biomarker³⁵

Saliva contains different types of molecules (like alpha-amylase, lactoferrins, transferrin etc).

Easily collectible: Patient is more co-operative while collecting sample in comparison with collection of hematological sample by venipuncture.

Non-surgical method: It is a non-surgical method for the diagnosis of disease.

Transmission of disease: There is little chance of transmission of diseases from the sample.

Easy to preserve: Saliva is normally used within 1 hour or refrigerated after collection.

Clotting: Saliva does not clot in comparison to the blood.

Possible mechanisms for the presence of biomarkers in saliva

The exact mechanism is unknown, but serum derived or locally produced may be the source of origin of nucleic acids and proteins (NAPs) (free from the cell) in saliva.³⁶

Salivary NAPs (derived from serum) may be secreted by acinar cells or come into the oral cavity by the help of cellular [Intracellular (active transport or passive diffusion)/extracellular (ultrafiltration through tight junctions)] routes or as constituents of crevicular fluid.

Table II

Types of salivary biomarkers for oral squamous cell carcinoma

Category	Salivary biomarkers
DNAs	P53, loss of heterozygosity in the combination of markers D3S1234, D9S156, and D17S799, mitochondrial DNAs (cytochrome oxidase I and II), hypermethylation of promoters in tumor suppressor genes: DAPK, DCC, MINT-31, TIMP-31, TIMP-3, p16, MGMT, CCNA1; mammary serine protease inhibitor (maspin), Telomerase activity
mRNAs	DUSP1 (dual specificity phosphatase 1), IL-8, IL-1b, H3F3A (H3 histone family 3A), S100P (S100 calcium binding protein P), SAT (spermidine/spermine N1-acetyltransferase EST), HOTAIR (HOX transcript antisense intergenic RNA)
Micro RNAs	miR-125a, miR-200a, miR-31
Proteins	P53, interleukin (IL) (8, 6, 1b), matrix metalloproteinase MMP (2, 9), transforming growth factor TGF-1, tissue polypeptide antigen (TPA), cancer antigen (CA-125), statherin, CD (59, 44), carcino embryonic antigen (CEA), Ki67, cyclic D1, cyfra 21.1, transferrin, alpha-amylase, tumor necrosis factor (TNF)-a, catalase, defensin-1
Oxidative stress-related molecules	Reactive nitrogen species (RNS) such as nitric oxide (NO), nitrites (NO ₂) and nitrates (NO ₃), peroxidase, glutathione S-transferase (GST), superoxide dismutase (SOD), 8-OHdG glutathione, malondialdehyde (MDA)
Salivary microbiota	Presence of human papilloma virus and Epstein-Barr virus (EBV), porphyromonas gingivalis, porphyromonas melaninogenica, <i>Streptococcus mitis</i> , <i>Candida albicans</i> and <i>Tannerella forsythia</i>

Table III

Techniques for extraction and analysis of salivary biomarkers

Techniques for extraction and analysis of salivary biomarkers	Categories of salivary biomarkers
Polymerase chain reaction (PCR)	DNAs
Quantitative polymerase chain reaction (qPCR)	
Microarrays	
Polymerase chain reaction (PCR)	mRNAs and microRNAs
Quantitative Polymerase chain reaction (qPCR)	
Microarrays	
High performance liquid chromatography (HPLC)	Proteins
Enzyme linked immunosorbent assay (ELISA)	
Radio-immunoassay two-dimensional gel electrophoresis (2DE)	
Mass spectrometry (MS)	
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)	
High performance liquid chromatography (HPLC)	Oxidative stress-related molecules
Calorimetric assay	

Normal epithelial or cancerous cells may release these salivary NAPs (free from the cell) which may be generated locally by cellular breakdown (necrosis, lysis, apoptosis or trauma). Patient with advanced stage cancer has shown that a large amount of DNA is present in the plasma which indicates that breakdown of cells (necrosis) lead to the presence of salivary NAPs (probable mechanism).

Availability of cellular NAPs in apoptotic bodies protects these molecules from degradation. Another strong possibility is the active release of cellular NAPs from the exosomes or micro-vesicles (come from cellular endoplasmic reticulum, membrane vesicles) while contact with the cell membrane. Cellular communication may also be maintained by exosomal contents [like mRNA, microRNA (miRNA) and proteins].^{19, 36, 37-48}

Salivary Biomarkers for Oral Cancer Detection

Salivary biomarkers can be a genetic marker, a protein or a metabolomic marker.⁴⁹ At the beginning, different types of salivary biomarkers (Table II) have been detected by applying several techniques (Table III) which can be remarkably raised in the saliva of oral cancer patient.⁵⁰⁻⁵² So, saliva is an important and acceptable tool for the diagnosis of oral squamous cell carcinoma due to its close relation with the oral lesion.

There are three levels for the diagnosis of oral squamous cell carcinoma on the basis of molecules;¹⁹ DNA of cell changes lead to a change of mRNA transcripts that induces alteration of protein levels intracellularly or extracellularly.

Salivary DNA as a biomarker

Each tumor has its own specific genetic materials which are present in the DNA of tumor cell. DNA biomarker is unique for an individual tumor which derives from the dead cells. It can be identified in the initial level of tumor formation as well as having a direct interaction on the development of a

neoplasm. DNA biomarkers have low tissue specificity.⁵³

Neoplastic cell's or dysplastic cell's DNA shows different types of alteration like deletion, point mutation, translocation, methylation, amplification, cyclin D1, microsatellite instability, epidermal growth factor (EGFR), and presence of human papilloma virus. Premalignant lesions with aneuploidy have more possibility to transfer into malignancy compare to lesions with normal DNA content.⁵⁴ DNA aneuploidy usually present in the late stage of carcinoma, lymph node metastasis and perineural invasion.⁵⁵ Therefore, invasiveness of the neoplasm can be portended by the help of DNA biomarkers of neoplasm.

When one of the chromosomal pair losses its genomic substance is known as loss of heterozygosity (LOH) which is a predictor for early assessment of the malignant conversion from precancerous lesions.⁵⁶ This aids physician to provide the treatment on the basis of the severity of the lesions. Oral carcinogenesis in the early stage is commonly associated with LOH in chromosome 3p, 9q, 13q and 17p.⁵⁷⁻⁵⁹ The chance of malignant conversion enhances 3.8-fold with allelic loss in chromosome 3p and 9p as well as 33-fold with LOH in chromosomes 4q, 8p, 11q, 13q and 17p besides the former.⁶⁰

Identification of exfoliated oral squamous cell carcinoma cells in the saliva by investigation of mitochondrial DNA mutations is also a good option as it can be found in 46 and 67% in saliva samples (collected by direct sequencing) with patient of oral squamous cell carcinoma.^{19, 61}

Cell cycle arrest and initiation of apoptosis in response to the DNA damage have been known to be regulated by p53 gene which is found on chromosome 17p and shows mutation in 50-70% of epithelial tumors such as oral squamous cell carcinoma,^{62, 63} 22% in pre-cancer cases such as actinic keratosis, keratoacanthoma and 20% in oral cancer cases are found to have p53 allele with LOH. p16, p27, p63, p73 genes are related to p53 gene which have been found to cause alteration of the cell cycle in certain degrees in oral cancer.⁶²

Promoter methylation associates with oral squamous cell carcinoma and the important genes are cadherin 1 (CDH 1), cyclin-dependent kinase inhibitor 2A (CDKN 2A), death-associated protein kinase 1 (DAPK 1) and methylguanine-DNA methyltransferase (MGMT).^{62, 64} Methylation of one or more of these genes (p16, DAP-K, MGMT) is noted in the oral squamous cell carcinoma and about 65% of matched saliva samples in oral squamous cell carcinoma patients are shown promoter hypermethylation.⁶²

20-40% of oral cancers have shown amplification and overexpression of c-MYCIN-MYC.⁶⁵ Another study showed about 30-50% of oral cancer patients are related to amplification of 11q13, containing HST1, 1NT2 and cyclin D oncogenes.⁶⁵ STAT 3 expressions and activation are noted in 82% patients of oral cancer associated with chewing tobacco in comparison with normal mucosa and premalignant lesions.⁶⁶ Cyclin D1 gene amplification is detected in poorly prognosis of oral squamous cell carcinoma.⁶⁷

The saliva of oral squamous cell carcinoma patient is associated with raised Ki67 marker and reduced 8-oxoguanine DNA glycosylase, phosphorylated-Src and mammary serine protease inhibitor (maspin).²³

The saliva of oral squamous cell carcinoma patient has shown 75% telomerase positivity indicating that telomerase detection could be used as an additional marker in oral squamous cell carcinoma.⁶⁸

Salivary RNA as a biomarker

The diagnosis of oral cancer using salivary RNA biomarker is thought to be part of the modern clinical approach. It was thought that degradation of salivary RNA occurs due to the presence of salivary RNAases and unlikely with passes of time, the truth brings that apoptotic bodies carry cell-free mRNA or may be actively released from exosomes or microvesicles in intact or fragment form.^{42, 47, 48, 69-72} It also found that saliva contains small RNA and miRNA which is thought to regulate the transcription.⁷³

The functions of enzymes are remarkably raised in a patient with oral squamous cell carcinoma and are thought to be related to seven types of mRNA of three different groups classified on the basis of severity of enhancement.⁷⁴ They are high, moderate and low up-regulated mRNA.

High up-regulated mRNA: a) IL8- takes part in replication, angiogenesis, immune response, chemotaxis, and cell cycle arrest.^{75, 76}

Moderate up-regulated mRNA: a) IL1 β - assists in signal transduction, proliferation, apoptosis and inflammation;^{75, 76} b) H3 histone, family 3A (H3F3A) takes part in DNA binding activity;⁷⁶ c) S100 calcium binding protein P assists for protein and calcium ion binding.⁷⁶

Low up-regulated mRNA: a) Dual specificity phosphatase 1 (DUSP1) takes part in transduction protein, signal modification and oxidative stress;⁷⁶ b) Ornithine decarboxylase antizyme 1(OAZ1) participates in polyamine biosynthesis;^{75, 76} c) Spermidine/spermine N1-acetyltransferase (SAT) (helps in enzyme and transferase activities) remarkably raises in oral squamous cell carcinoma patients.^{75, 76}

Small (19-25 nucleotides) non-coding

(RNA molecule that is not translated into a protein) RNA molecules are called miRNA which is related to post-transcriptional regulation by the help of RNA-induced silencing complex. They are present in the salivary cell-derived exosomes and are well-known to help in cell growth, differentiation, pathogen-host interactions and stress responses, apoptosis, and immune function. Their morphological presentation (from 10 to over 100 fold) varies with different types of cancer cells in comparison to the normal cell. A study shows that the saliva of oral cancer patient contains remarkably low level of miRNAs (miR-200a and miR125a, called tumor suppressors) in relation to the control group and also found that salivary miR-31 level is remarkably raised in all stages of oral malignancy as well as found higher in the saliva than the blood due to derive from the oral neoplasm.⁷⁷

Salivary protein as a biomarker

Differentiation of antigens of particular healthy tissue is known as protein biomarker which indicates the definite stage of its maturation. They are derived from living cells and present with high tissue specificity and can even be found in certain pathological conditions.⁵³ As a prognostic biomarker, sensitivity and specificity of salivary protein biomarkers remain moderate in level.¹⁹ In cancer research, special attention has been made by carbonylation (means oxidative damage to proteins) due to its irreversible and irreparable nature, which turns into the cytotoxic condition and causes cancer.⁷⁸ Studies have shown that salivary carbonyls of oral squamous cell carcinoma patient are remarkably raised (246%) because of invading free radical into the exposed epithelial cells.²³

Different types of metalloproteinases like MMP-2, MMP-9, and MMP-11 are noticeably changed in the oral squamous cell carcinoma.^{23,78,79} Degradation of different types of collagen (type IV, V, VII, X), elastin and fibronectin are associated with MMP-9.²³ Increased level of MMP-9 can be found in stromal cells surrounding metastatic neoplasms.^{80,81} MMP-9 polymorphism has a strong relation with high-risk for developing oral squamous cell carcinoma.⁸² Studies suggested that MMP-9 of oral squamous cell carcinoma patient is raised 39% with 100% sensitivity and 79% specificity.²³

Interleukin (IL)-6 and IL-8 are recognized as necessary mediators for developing cancer and strong activators for apoptosis as well as anti-apoptotic signalling cascade, therefore help in early diagnosis of oral squamous cell carcinoma and oral pre-malignancies.⁸³ Most of the normal cells and malignant epithelial cells contain cytoskeletal intermediate filaments known as cytokeratins.⁸⁴ The activated protease of malignant epithelial cells enhances the degeneration of cytokeratin free filaments into the blood. Salivary cyfra 21-1 is found

to be raised in the patient with oral squamous cell carcinoma.⁸⁴⁻⁸⁶ Antimicrobials and cytotoxic properties are exhibited by salivary defensins which are located in azurophil granules of polymorphonuclear leukocytes and study got that salivary defensin-1 is increased in oral squamous cell carcinoma.⁸⁸⁻⁹⁰ Other types of salivary biomarkers such as inhibitor of apoptosis (IAP),⁹¹ lactate dehydrogenase (LDH),⁸⁶ SCC-antigen,^{87,92} carcino-antigen (CA19-9),^{87,92} CA128,⁹² carcino-embryonic antigen (CEA),^{87,93} immunoglobulin G (IgG),⁸⁶ s-Ig A,⁷⁸ insulin growth factor (IGF),⁷⁸ serum tumor marker (CA125),^{87,94} tissue polypeptide specific antigen (PPS),^{87,95} and reactive nitrogen species (RNS)⁹⁶ which are noticeably altered with patients of oral squamous cell carcinoma in comparison with the control group.

Molecules related to oxidative stress

Diagnosis of oral cancer can be assessed by oxidative stress. Salivary reactive nitrogen species are remarkably raised with the patient of oral cancer in comparison to the control group. Meanwhile, other salivary anti-oxidants are noticeably decreased (because of utilization), that lead to the oxidative destruction of DNA and proteins, thus helps in oral cancer detection.¹⁶

The salivary microbiota

Overgrowth of oral microflora may occur due to some alteration in the diet, medication, habit, and host immune status which act as a predisposing factor for the development of the disease. The levels of *Porphyromonas gingivalis*, *Candida albicans* and *Tannerella forsythia* of the cancer group are enhanced noticeably in comparison to the control group.⁹⁷ *P. gingivalis*, *Streptococcus mitis* and *P. melaninogenica* are increased in the saliva of oral squamous cell carcinoma patient which indicate that salivary microbiota can act as a diagnostic marker for oral squamous cell carcinoma.⁹⁸

Candidal carriage in the saliva of oral squamous cell carcinoma patients is elevated in comparison with control groups which suggest that salivary analysis of *Candida* species may be effective for diagnosing the oral cancer.⁹⁷

The identification of human papilloma virus and Epstein-Barr virus (EBV) genomic sequence has played a vital role for the diagnosis of oral squamous cell carcinoma as well as for the assessment of the progression of disease.⁹⁹

Conclusion

Oral cancer can be diagnosed by using salivary diagnostics tools which are vast, perplexing and brightening area of scientific research. Salivary biomarkers are noticeably elevated in pathological

conditions which are really inspiring to think about the advantages of saliva as an important diagnostic method alternative to hematological examination. Myriad labors from clinicians and researchers are necessary to bring salivary diagnostic method into clinical as well as commercial reality for fighting against oral cancer.

Conflict of interest

The author(s) declare that they have no conflict of interest.

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