

Proteomics and Oral Cancer: The Road Less Travelled

Dr. Kumar Gaurav Chhabra¹, Sayali Limsay², Radhika Kulkarni³, Nandini Hake⁴, Dr. Gargi Nimbulkar⁵, Dr. Amit Reche⁶

¹Reader, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, (Meghe)Wardha, 442001, Maharashtra, India

²Asst. Resident, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, (Meghe)Wardha

³Asst. Resident, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, (Meghe)Wardha

⁴Nandini Hake, Asst. Resident, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, (Meghe)Wardha

⁵Dr. Gargi Nimbulkar, Assistant Professor, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University); Sawangi, (Meghe)Wardha, 442001, Maharashtra, India

⁶Dr. Amit Reche, Assistant Professor, Department of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, (Meghe)Wardha, 442001; Maharashtra, India

Email: ¹rajsushil.chhabra@gmail.com, ²sayalilimsay@gmail.com;
³radhikakulkarni1196@gmail.com, ⁴nandineehake02@gmail.com,
⁵garginimbulkar@gmail.com

Review Article Conflict of Interest: None

Abstract

The oral squamous cell carcinoma (OSCC) is evaluated as the eighth driving reason for malignant growth all inclusive. OSCC shows high predominance and grimness, with the death pace of 145,000 passing for every year universally. The result of the treatment and anticipation of oral cancerous growth relies for the most part upon early finding. For the high morbidity of the oral cancer, deferred identification is probably going to be an essential explanation and this backing the basic requirement for sensitive biomarkers to improve early recognition of oral malignant growths. Along these lines, early location and counteraction of oral cancerous growth is one of the destinations set by World Health Organization (WHO). Abnormal cellular products from malignant cells can be recognized and estimated in different body fluids, for example, blood, urine and saliva. Saliva can be utilized to identify biomarkers explicit for oral cancer as it is anything but difficult to acquire and non-intrusive, additionally due to its immediate contact with oral cancer, salivary assessment for discovery of oral cancer can show the best benefit. Biomarkers are utilized for patient assessment in various clinical settings. They are likewise utilized for assessing disease risk, screening for mysterious primary cancers, differentiating benign from malignant findings/one sort of malignancy from another, determining prognosis,

acting as indicators/screening, and observing disease status. Salivary biomarkers can possibly recognize normal biological, pathological as well as pharmacological response to treatment. Huge endeavours from scientists and clinicians are significant so as to transform salivary diagnostics into clinical and commercial reality and in this way helping in battling oral disease. This article gives survey of salivary proteomic biomarkers, strategies for examination of salivary proteomic biomarkers and their clinical significance in early discovery of oral cancers.

Key words: Oral Squamous Cell Carcinoma, Proteomics, Saliva

1. INTRODUCTION:

Being the fifteenth generally pervasive, Oral cancer has the normalized occurrence pace of 3.9 per one lakh populaces around the world (1). This feared malignancy turns into the significant health problem because of increasing patterns in more youthful populace. The India represents 33% of the worldwide weightage of this malignancy (2). The age normalized frequency pace of oral cancer is 12.6 per one lakh populace in India, also there is marked increment in the occurrence pace of oral cancer has been accounted for in ongoing years (3). For the high morbidity of the oral cancer, deferred identification is probably going to be an essential explanation and this backing the basic requirement for sensitive biomarkers to improve early recognition of oral malignant growths. As of now, oral cancerous growth determination is subject to an intensive oral assessment, normally by a dental specialist or other qualified health care provider, for potential signs and side effects of the disease. On the off chance that an assessment shows a abnormal area in the oral cavity, a little tissue biopsy might be evacuated for a pathologist to check for cancer cells under a microscope. Researchers are scanning for biomarkers in saliva, which is anything but difficult to get and furthermore non-obtrusive for the detection of oral cancer. Mitochondrial DNA change and variant promoter hypermethylation of malignancy related genes are normal in head and neck/oral cancer. Discovery of these hereditary modifications in saliva might be utilized for analysis and observing of the disease (4) (5)

2. ADVANTAGES OF SALIVA OVER OTHER NON-OBTRUSIVE BIOMARKERS

In view of its immediate contact with oral cancer lesion, salivary assessment for location of oral cancer can show the best benefit. The utmost significant reason for choosing saliva as a assessment aid is that it additionally contains the slaughtered cells in oral cavity. Because of this, saliva can be the chief alternative of screening and discernation of possible biomarkers for head and neck cancer. Saliva samples are normally safe to deal with. Biomolecules present in the saliva are known to hinder the HIV infection. In this manner, odds of transference from saliva are extremely little when contrasted with transference from blood tests. When contrasted with blood tests, salivary specimens are anything but difficult to store, as saliva doesn't clump. Non-obtrusive saliva securing is effortless and should be possible by anybody and this diminishes nervousness and inconvenience of patients. Cell free saliva contains a great many proteins just as mRNA and microRNA transcripts, and metabolites having vast scope of biological activities. Blood DNA which is obtained from an assorted populace of defence cells relies upon singular condition of immune activation, while salivary DNA is extracted from a analogous sub populace of polymorphonuclear white blood cells and epithelial cells of the oral cavity (6). Saliva comprises reduced foundation of typical substances and inhibitory substances just as less complexes than blood. Salivation has numerous advantages over serum and tissues, including easy to collect, storage and dispatching, cost adequacy, easy accessibility of huge sample volume for investigation and

continued examining for observing over time (7)

3. SALIVARY BIOMARKERS

Neoplastic procedure gives/delivers a few irregular cell items. They can be recognized in different body fluids and on the surface of tumour cells either by biochemical techniques or by immunohistochemistry. These items which are identified and estimated are known as "tumor markers"

A tumor marker or biomarker is available in or produce by a tumor or tumours' host in light of the tumour's essence. They are valuable in the separation a tumor from typical tissue or in deciding the presence of a tumor dependent on estimation in blood or discharges. The distinctive biological attributes of tumor cells are the capability of invasion, metastasis, boundless proliferation, avoidance of apoptosis and angiogenesis. These procedures are completely interceded by complex molecular pathways and any of these can be potential tumor markers. A few salivary tumor markers are seen as altogether increased in the salivation of oral cancer patients. Salivary markers for the analysis of oral cancer can be found in 3 levels; changes in the cell Deoxyribonucleic corrosive (DNA) which causes modification mRNA transcripts which further prompts adjusted protein levels intracellularly, on the cell surface or extracellularly (8)

Salivaomics is an expansive collection of technologies which is utilized to investigate various kinds of molecules present in saliva. It incorporates genome and epigenome (the investigation of genes and their methylation), transcriptomics (the investigation of messenger RNA (mRNA) inside cells or organisms), metabolomics (the investigation of worldwide metabolite profiles in a framework), proteomics (the investigation of proteins) and microbiota (the investigation of microbiology). The salivary gene sequence comprises human as well as microbial DNA. The amount as well as the nature of salivary DNA are very acceptable: Saliva tests additionally produce sufficient DNA for sequencing clusters and polymerase chain response (PCR) examines. Both salivary genome and epigenome are assayable by a different aggregation of biomolecular procedures, involving methylation clusters, PCR and quantitative PCR (qPCR)based genotyping. The salivary proteome is containing the whole protein substance of the oral cavity. Salivation comprises more than 2000 proteins and peptides which are associated with a large number of various biological capacities in the oral cavity are available in the saliva. When contrasted with plasma proteins, 25 percent of the entire proteins are found in salivation. Proteomic investigation in salivation has particular focal points over blood, particularly for low-abundance proteins (9)

Biomarkers are utilized for patient assessment in various clinical settings. They are likewise utilized for assessing disease risk, screening for mysterious primary cancers, differentiating benign from malignant findings/one sort of malignancy from another, determining prognosis, acting as indicators/screening, and observing disease status. They can be utilized to either identify recurrence or decide progression/reaction to treatment. The assessment of a danger of patient of developing oral cancer is useful if chance reduction procedures or screening are viable. At the point when these methodologies applied to high risk groups are more productive than wholesale application to the whole population (10)

Circulating tumor markers for OSCC have been investigated in different studies and these markers have demonstrated moderate sensitivity and specificity esteems. Examiners have mostly utilized two methodologies for the investigation of markers of malignancies: Epithelial dysplasia on one hand and oral cancer on the other are characterized by the nearness or nonattendance or the pattern in which the related marker has been circulated. The marker is helpful if the response pattern in epithelial dysplasias is like that in carcinomas as well as if the abnormal response pattern is identified with the evaluation of epithelial dysplasia in a positive way. These organic markers for deciding future disease advancement

in oral pre-malignant lesions can be extensively partitioned into (a) genomic markers, including DNA content (ploidy) chromosome variation, and changes in articulation of oncogenes and tumor suppressor genes; (b) multiplication markers; and (c) separation markers, including keratins and sugar antigens which will be discussed(8)

Clinical significance of various salivary biomarkers(11)(12)(13)(14)(15)(16)(17)(18)(19)

Biomarkers	Clinical significance
Cyc D1, Ki67 LDH, MMP9 OGG1, Maspin	In oral cancer patients these were considerably altered.
IL-1, IL-6 IL-8, TNF-α	The proinflammatory and proangiogenic cytokines apparently was expanded in head and neck cancer and precancerous individuals.
Telomerase	It is seen as elevated in saliva of head and neck cancer individuals.
p53 autoantibodies	Its presence in saliva and serum of oral cancer patients can be utilized as a non-obstructive procedure for the identification of subset of tumors.
Reactive nitrogen species	Its level has demonstrated to be changed in saliva of oral carcinoma cases which shows the chance of direct association between salivary free radicals, cell reinforcements and oral carcinoma.
IL-6, TNF-α	Elevated levels of these variables in salivation of patients with oral leukoplakia when contrasted with the controlled group.
HPV	Its presence in salivary flushes shows chances for molecular screening of HPV related oral cancer.

M2BP, MRP14 CD59, Profilin 1 Catalase	Seen as modified in salivation of oral carcinomacases when contrasted with healthy people.
Actin, myosin	Can be utilized to separate precancerous and malignant patients.
Transferrin	Its level in saliva of oral cancerous growth patients can be utilized to associate with size furthermore, stage of the tumor.
Salivary mRNA	With the assistance of RT- PCR, from one droplet of saliva large number of mRNAs can be made to analyse.
DNA Hypermethylation	Set of specific cancer related genes can be made by methylation array of saliva.
Salivary mRNA and protein	With the help of EC sensor, level of these biomarkers in saliva can be identified which shows considerable difference in healthy individual and oral cancer patients.

Liao et al. in 2000 distributed the first investigation of saliva as a demonstrative apparatus for oral cancer identification. As indicated by the creators, exon 4, codon 63 of the p53 quality was transformed in salivary DNA from sixty two percent of oral cancer cases. Notwithstanding these autoantibodies against p53, the deviantly communicated protein in cases with oral cancerous growth has been found in both saliva just as serum. TP53 was the main quality which has comparative occurrences of loss of heterozygosity (LOH) and mutations. LOH was appeared to happen all the more much of the time in oral cancerous

growth patients when contrasted with quality transformations.

Oxidative pressure assumes a significant job in movement of oral carcinoma. Bahar et al. recorded every single salivary cell reinforcement were evidently lesser in the oral carcinomacases when contrasted with the controls aside from salivary responsive nitrogen species which were significantly higher. This expansion in receptive nitrogen species may prompt the utilization and decrease of salivary cell reinforcements bringing about the oxidative harm to DNA and proteins, and perhaps prompting movement of oral carcinoma.

Shiptzer et al. discovered that expanded salivary degrees of cell cycle administrative proteins involving Cyclin D1 and ki67, glycolytic compound lactate dehydrogenase (LDH), framework metalloproteinase (MMP)- 9, just as decrease in DNA fix catalyst, 8-oxoquanine DNA glycosylase (OGG1) and Maspin, a tumor silencer protein in oral cancerous growth patients. Sato et al. reported that there is significantly expanded interleukin (IL)- 6 levels in saliva of oral carcinoma cases than controls.

Brailo et al found that there are adjustments in salivary IL-6 and tumor corruption factor alpha (TNF-an) in oral leukoplakia cases. They likewise saw that salivary IL-6 and TNF-a levels were markedly higher in oral leukoplakia cases when contrasted with the sound people. IL-6 inactivates p53 tumor silencer quality by supporting the hypermethylation of its advertiser district which is bringing about concealment of apoptosis and uncontrolled cell expansion. TNFa initiates NFjB translation factor which further invigorates cell multiplication and squares apoptosis. Notwithstanding that, it improves discharge of proinflammatory cytokines. Rhodus et al. discovered that there are significantly higher salivary degrees of IL-1, IL-6, IL-8 and TNF-an in oral cancer patients when contrasted with the patients with dysplastic oral sores and control. IL-6 is likewise related with the repeat of oral cancer. Zhong et al. detailed 75% positive articulation of telomerase in salivation of oral carcinoma cases recommending its convenience as a strong marker to analyse oral carcinoma as well as furthermore proposed that human telomerase invert transcriptase (hTERT) investigation might be a possible biomarker for the analysis of oral carcinoma. Telomerase is a ribonucleoprotein which helps in prolonging rehash succession toward the finish of the chromosomes.

The ongoing examination points towards distinguishing the human papilloma infection (HPV) in salivation, as the causative factors in oral carcinoma. The frequency of HPV in cases managed for oral cancerous growth is evaluated, which is more than 45% (20)

4. INVASION AND METASTASIS RELATED SALIVARY BIOMARKERS

Oral malignant growth has a significant level of intrusion and a high pace of metastasis to cervical lymph nodes. Gelatinases; particularly, gelatinase A for example MMP-2 and gelatinase B for example MMP-9 were appeared to assume a remarkable job in attack as well as in metastasis as they corrupt Type IV collagen which is a significant part of the basement membrane layer. It is demonstrated that salivary degrees of MMP-2 and MMP-9, the intrusion and metastasis related markers additionally have an significant job in the pathological process of oral cancer. It is seen that both active just as latent types of salivary MMP-2 and MMP-9 were exceptionally elevated in oral carcinoma cases when contrasted with the controls. The receiver characteristic curve (ROC) analysis uncovered that each types of MMP-2 and MMP-9 segregate between oral malignancy cases and controls (20) (16) (21) (22)

5. METHODS OF ANALYSIS OF SALIVARY PROTEOME

Protocol of submission of saliva- To avoid bacterial contamination saliva should be collected and refrigerated at 4 degree C and further operation should also be done in low temperature within 3 to 6 hours (23). To impede bacterial growth, it is suggested to add sodium azide in

saliva (24). Addition of sodium azide may cause intrusion in immunoassays with horseradish peroxidase. To avoid protein denaturation, protease inhibitors and stabilizers, for example, aprotinin, leupeptin, EDTA can be applied.

There are two types of analytical methods for salivary proteomes that are top-down and bottom-up (25). Top-down method of analysis mainly involves analysis of proteomes which are naturally present on the other hand bottom up proteomics involves analysis of digested salivary proteins. In a top-down type of analysis, two-dimensional gel electrophoresis (2DGE) is the basic technique which allows separation of complex protein mixtures (26). There are some limitations, they are-(1) small peptides or proteins having mere acidic or basic isoelectric points can drift outside its analysis ranges (27). (2) by this technique protein which are less in concentration can get obscure by the protein which are high in concentration. (3) this process undergoes from many discrepancies such as abnormal migration, gel preparation and staining protein isoforms.

Mass spectrometry (MS) allows to detect salivary proteomes which are in stage of expression and post translational modification. This technique is highly sensitive as well as fast. Surface improved laser desorption ionization (SELDI), framework helped laser desorption ionization (MALDI) or time-of-flight (TOF) can be used to combine with MS to measure intact protein or peptides. SELDI-TOF-MS is involved in purification of sample, desorption and separation of protein in protein chip. For initial profiling MALDI-TOF-MS can be used which has higher sensitivity. Further the identification can be done with the help of HPLC-MS.

Isotope-coded partiality labels (ICAT), isotope labels for relative and outright measurement (iTRAQ), total evaluation of proteins utilizing interior principles (AQUA) and stable isotope marking by amino acids in cell culture (SILAC) are chemical labelling procedures which are also be used to detect variation in concentration of salivary protein in changeable stages (28) (23) (25)

6. DISADVANTAGED POPULATIONS AND THE ROLE OF SALIVARY BIOMARKERS.

Cancer is one of the most widely recognized reasons for morbidity and mortality these days. Around 43% of deaths due to cancer, it has been assessed, are because of tobacco use, diets which are unhealthy, consumption of liquor, inactive ways of life and disease. Low-pay and less privileged groups are commonly increasingly presented to different avoidable hazard factors, for example, natural cancer-causing agents, liquor, infectious agents and the utilization of tobacco. Additionally, these groups have less access to the health care services and health education. Both of these are important to enable and teach them to improve choices to ensure and improve their own health. Tobacco and liquor are viewed as a portion of the significant hazard factors for oral disease. The dangers, ascribed to populace, of smoking and liquor utilization have been assessed to 80% for guys, 61% for females, and 74% generally speaking. The International Agency for Research on Cancer has as of late affirmed the proof that smokeless tobacco causes oral malignant growth. Oral disease is preventable through hazard factors intervention (29)

Saliva is a watery substance present in the mouth and contains useful segments that have been utilized as diagnostic markers for human disease. Oral cancer has been demonstrated to be reflected diagnostically in salivation. Saliva is a reasonable, non-intrusive and available tool in the diagnosis, prediction of prognosis, as well as checking of the patients' post treatment status in oral cancer (8)

Thus various efforts in creating proper awareness and educating the disadvantaged populations about the usefulness of the salivary biomarkers, through health education programmes along with the combined efforts of dentists explaining the importance of early detection of cancer with the help of these salivary biomarkers and how this can possibly

reduce the mortality, morbidity and economic setbacks caused to them during the course of the treatment of this disease, should be undertaken

The Ki67 LI, Alpha Smooth Muscle Actin (SMA) and MVD can be used as predictive markers of clinical outcome of Oral Squamous Cell Carcinoma – Submucous Fibrosis. Ki-67 is a great immunohistochemical marker to decide multiplying cells of the tumour. It is communicated in every dynamic period of the cell cycle (G1, S, G2, and M stage) aside from in resting cells (peaceful cells – G0 stage). The monoclonal Ki-67/MIB-1 antibody responds with the atomic Ki-67 antigen communicated in multiplying cells. Its appearance reflects tumour expansion and has been found to show tumour hostility, tumour metastasis, and known to anticipate outcome result in numerous human malignancies, for example, CNS tumours, lymphoproliferative illnesses, connective tissue tumours, and mammary gland tumours. (30)(31)

Most of the invasive oral carcinomas are gone before by clinical precancerous lesions and conditions, for example, oral leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis (OSMF). These stay in preinvasive stage for a considerable length of time and the carcinogenic modifications stay lethargic and not promptly conspicuous on clinical and histopathologic assessment. (32)

Customarily, cervical nodes are assessed by clinical assessment, yet this has been accounted for in the writing to have a low affectability and particularity. Computed Tomography, Magnetic Resonance Imaging, and Ultra-sonography are touchy strategies; however, they need particularity. Either frozen section investigation or touch imprint cytology can achieve intraoperative appraisal of lymph node. (33)

7. CONCLUSION

As of now, the research field concerned with saliva is quickly developing and progressing because of the utilization of novel methodologies, for example, metabolomics, genomics, proteomics and bioinformatics. It has been shown that salivation involves fundamentally progressively clinical information isolated from its helpful worth. Because of its closeness to the oral cavity as well as non-intrusive method of collection, salivary examination can be the prime method as an essential investigation for oral carcinoma. (20)

Saliva additionally contains cells which can be stumble from the carcinogenic tissue in the oral cavity (34)

It is strongly prescribed to develop specific biomarkers for oral disease screening on the grounds that early identification of oral malignant growth will assist with diminishing patient morbidity and mortality (10)

The field of salivary genomics and proteomics biomarker investigation is exceptionally encouraging and may improve the field of oral cancer diagnosis further empowering clinicians to screen patients' saliva for diagnosis and determining the prognosis of oral cancer in this manner propelling the clinical endeavours to beat the seriousness of the illness. Huge endeavours from scientists and clinicians are significant so as to transform salivary diagnostics into clinical and commercial reality and in this way helping in battling oral disease (20)

Acknowledgement

The investigators acknowledge the support of Dean, SPDC and Department of Public Health Dentistry in the execution of this project..

REFERENCES

- [1]. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010 Dec 15;127(12):2893–917.
- [2]. Subramanian S, Sankaranarayanan R, Bapat B, Somanathan T, Thomas G, Mathew B, et al. Cost-effectiveness of oral cancer screening: results from a cluster randomized controlled trial in India. *Bull World Health Organ*. 2009 Mar;87(3):200–6.
- [3]. Misra C, Majumder M, Bajaj S, Ghosh S, Roy B, Roychoudhury S. Polymorphisms at p53, p73, and MDM2 loci modulate the risk of tobacco associated leukoplakia and oral cancer. *Mol Carcinog*. 2009 Sep;48(9):790–800.
- [4]. Micrometastasis - Google Books [Internet]. [cited 2020 May 16]. Available from: <https://books.google.co.in/books?id=8V4ECAAAQBAJ&pg=PA42&lpg=PA42&dq=>
- [5]. Sidransky+D.+Nucleic+acid-based+methods+for+the+detectionofcancer.+Science1997;278:1054%5E9.&source=bl&ots=swPad07vsC&sig=ACfU3U3Y07z7F6n4RDDe3Ob1CW81K05ppw&hl=en&sa=X&ved=2ahUKewjsrPbg7rfpAhXYfH0KHxDeUQ6AEwAHoECACQAQ#v=onepage&q=Sidransky%20D.%20Nucleic%20acid-based%20methods%20for%20the%20detectionofcancer.%20Science1997%3B278%3A1054%5E9.&f=false
- [6]. Righini CA, de Fraipont F, Timsit J-F, Faure C, Brambilla E, Reyt E, et al. Tumor-specific methylation in saliva: a promising biomarker for early detection of head and neck cancer recurrence. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2007 Feb 15;13(4):1179–85.
- [8]. Chattopadhyay I, Panda M. Recent trends of saliva omics biomarkers for the diagnosis and treatment of oral cancer. *J Oral Biosci*. 2019 Jun 1;61(2):84–94.
- [9]. Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. *J Am Dent Assoc*. 2006 Mar 1;137(3):313–21.
- [10]. An insight into salivary markers in oral cancer [Internet]. [cited 2020 May 14].
- [11]. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3760349/>
- [12]. Wang X, Kaczor-Urbanowicz KE, Wong DTW. Salivary biomarkers in cancer detection. *Med Oncol*. 2016 Dec 10;34(1):7.
- [13]. Santosh. A review on oral cancer biomarkers: Understanding the past and learning from the present [Internet]. [cited 2020 May 14]. Available from: <http://www.cancerjournal.net/article.asp?issn=0973-1482;year=2016;volume=12;issue=2;spage=486;epage=492;aulast=Santosh>
- [14]. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detect Prev*. 2005;29(1):42–5.
- [15]. Zhong L-P, Chen G-F, Xu Z-F, Zhang X, Ping F-Y, Zhao S-F. Detection of telomerase activity in saliva from oral squamous cell carcinoma patients. *Int J Oral Maxillofac Surg*. 2005 Jul;34(5):566–70.
- [16]. Warnakulasuriya S, Soussi T, Maher R, Johnson N, Tavassoli M. Expression of p53 in oral squamous cell carcinoma is associated with the presence of IgG and IgA p53 autoantibodies in sera and saliva of the patients. *J Pathol*. 2000 Sep;192(1):52–7.
- [17]. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. *Cancer*. 2007 Jan 1;109(1):54–9.
- [18]. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. - PubMed - NCBI [Internet]. [cited 2020 May 16].
- [19]. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/16324876>

- [20]. Activation of MMP-2 and MMP-9 in patients with oral squamous cell carcinoma. - PubMed - NCBI [Internet]. [cited 2020 May 16]. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/15844188>
- [21]. Viet CT, Jordan RCK, Schmidt BL. DNA promoter hypermethylation in saliva for the early diagnosis of oral cancer. *J Calif Dent Assoc.* 2007 Dec;35(12):844–9.
- [22]. Wei F, Patel P, Liao W, Chaudhry K, Zhang L, Arellano-Garcia M, et al. Electrochemical sensor for multiplex biomarkers detection. *Clin Cancer Res Off J Am Assoc Cancer Res.* 2009 Jul 1;15(13):4446–52.
- [23]. Zimmermann BG, Wong DT. Salivary mRNA targets for cancer diagnostics. *Oral Oncol.* 2008 May 1;44(5):425–9.
- [24]. A Review on Salivary Genomics and Proteomics Biomarkers in Oral Cancer | SpringerLink [Internet]. [cited 2020 May 14]. Available from: <https://link.springer.com/article/10.1007/s12291-011-0149-8>
- [25]. Clinical significance of MMP-2 and MMP-9 in patients with oral cancer. - PubMed - NCBI [Internet]. [cited 2020 May 16]. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/17252594>
- [26]. Matrix Metalloproteinases and Their Inhibitors: Correlation with Invasion and Metastasis in Oral Cancer [Internet]. [cited 2020 May 16]. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3001841/>
- [27]. Potential uses of human salivary protein and peptide analysis in the diagnosis of disease. - PubMed - NCBI [Internet]. [cited 2020 May 16]. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21774913>
- [28]. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systematic review [Internet]. [cited 2020 May 16]. Available from: https://hrca.hr/index.php?show=clanak&id_clanak_jezik=206213
- [29]. The human salivary proteome: a critical overview of the results obtained by different proteomic platforms: Expert Review of Proteomics: Vol 9, No 1 [Internet]. [cited 2020 May 16]. Available from: <https://www.tandfonline.com/doi/abs/10.1586/epr.11.77>
- [30]. Rubporn A, Srisomsap C, Subhasitanont P, Chokchaichamnankit D, Chiablaem K, Svasti J, et al. Comparative Proteomic Analysis of Lung Cancer Cell Line and Lung Fibroblast Cell Line. *Cancer Genomics - Proteomics.* 2009 Jul 1;6(4):229–37.
- [31]. Two-dimensional gel electrophoresis in proteomics: Old, old fashioned, but it still climbs up the mountains - Rabilloud - 2002 - PROTEOMICS - Wiley Online Library [Internet]. [cited 2020 May 16]. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/1615-9861%28200201%292%3A1%3C3%3A%3AAID-PROT3%3E3.0.CO%3B2-R>
- [32]. Li Q, Ouyang X, Chen J, Zhang P, Feng Y. A Review on Salivary Proteomics for Oral Cancer Screening. *Curr Issues Mol Biol.* 2020;47–56.
- [33]. Petersen PE. Oral cancer prevention and control--the approach of the World Health Organization. *Oral Oncol.* 2009 May;45(4–5):454–60.
- [34]. Gadbaile AR, Chaudhary MS, Sarode SC, Gondivkar SM, Belekar L, Mankar- Gadbaile MP, et al. Ki67, CD105 and α -smooth muscle actin expression in disease progression model of oral submucous fibrosis. *J Invest Clin Dent.* 2019 Nov;10(4):e12443.

- [35]. Laishram. To assess the utility of proliferative marker Ki-67 in surface epithelial ovarian tumor [Internet]. [cited 2020 Jun 18]. Available from: <http://www.journaldmims.com/article.asp?issn=0974-3901;year=2019;volume=14;issue=1;spage=6;epage=10;aualast=Laishram>
- [36]. Alka. Immunohistochemical analysis of tumor-associated stroma in oral squamous cell carcinoma with and without preexisting oral submucous fibrosis [Internet]. [cited 2020 Jun 18]. Available from: <http://www.journaldmims.com/article.asp?issn=0974-3901;year=2017;volume=12;issue=3;spage=170;epage=176;aualast=Alka>
- [37]. Agarwal A, Bhola N, Kambala R, Borle RM. Touch Imprint Cytology: Can It Serve as an Alternative to Frozen Section in Intraoperative Assessment of Cervical Metastasis in Oral Squamous Cell Carcinoma? *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg*. 2019 May;77(5):994–9.
- [38]. Wu J-Y, Yi C, Chung H-R, Wang D-J, Chang W-C, Lee S-Y, et al. Potential biomarkers in saliva for oral squamous cell carcinoma. *Oral Oncol*. 2010 Apr 1;46(4):226–31.