ejpmr, 2017,4(6), 261-267

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A NEW TOOL FOR DIAGNOSIS OF ORAL CANCER -SALIVARY TUMOR MARKERS: A REVIEW ARTICLE

[1]Dr. Amritaksha Bhattacharyya, [2]*Dr. Bhavana Gupta and [3]Dr. Anil Singh.

[1]Senior Lecturer, Department of Oral Pathology, Maharana Pratap Dental College, Kanpur.(U.P).
[2]*Senior Resident, Department of Oral Pathology, KGMU, Lucknow. (U.P).
[3]Professor and Head, Department of Oral Pathology, Saraswati Dental College Lucknow.

***Corresponding Author: Dr. Bhavana Gupta**

Senior Resident, Department of Oral Pathology, KGMU, Lucknow. (U.P).

Article Received on 17/04/2017 **Article Revised on 07/05/2017** **Article Accepted on 27/05/2017**

ABSTRACT

The components present in the saliva "epitomize the body's health condition". The saliva acts as an promising body fluid for recognition of clinical diseases with several advantages for disease diagnosis and prognosis. Oral and systemic diseases may influence the quantity and composition of saliva that is formed. The protein resultant from the living cancer cells along with DNA and RNA and inflammatory cells then can be expediently derived from saliva. The genomic and proteomic studies are done to recognize the possible biomarkers in body fluids like saliva and blood for the better diagnosis and prognosis of Oral cancers. This manuscript reviews the recently recognized different salivary biomarkers that can be used as essential markers for the diagnosis and prognosis of various carcinomas of oral cavity.

KEYWORDS: Oral cancer, Saliva, Biomarker, Oral carcinoma.

INTRODUCTION

In the midst of all the cancers, oral cancer is known to be the sixth most common cancer in the world.[1] About 90% of oral cancers are supposed to be squamous cell carcinoma (SCC).[2]

Tobacco along with betel quid habbits and the regular drinking of alcoholic beverages are known to be the main risk factors for the progress of oral squamous cell carcinoma (OSCC). The frequency and occurrence of oral cancer is established highest in the asia wherever the habits of chewing tobacco, betel quid and areca-nut are very prevalent.[1]

Oral cancer often present with symptoms at a late stage. High recurrence rate especially in those with nodal metastasis and low overall five year survival rates have remained unchanged during the past few decades. The high morbidity rate of oral cancer patients is primarily related to delayed detection of the condition, and this supports the imperative need for sensitive techniques of early detection of OSCC.[3]

The gold standard for oral cancer diagnosis is based on expert clinical examination and histological analysis of suspicious areas.[4] Initial diagnosis depends on a thorough oral examination, usually by a dentist or other qualified health care provider, for probable signs and symptoms of the oral diseases followed by the confirmatory histopathological examination of the tissue biopsy from the suspected area.[5]

In an attempt to aid the early diagnosis of oral cancer different screening techniques are used.

Mainly oral cancer screening programs contain the simple visual examination,[6-7] whereas others endeavor to employ the use of toluidine blue,[8-9] brush biopsy (exfoliative cytology),[10-11] chemiluminesce,[12-13] and fluorescence imaging. The later three screening methods are indeed deal with the diagnosis of different oral lesions which have been previously detected by dentists or other clinician but a authoritative diagnosis can only be made by a tissue biopsy.[14]

The genetic aberrations in the cancer cells guide to altered expression patterns of genes and proteins, which can be known previously results in different cancer phenotypes. Changes that arise exclusively or preferentially in cancer, compared with normal tissue of the same origin known as tumor markers, can be used as molecular biomarkers.[15]

Nevertheless, cancer cell lines which are present obtained from biopsy specimens from invasive and metastatic cancers, are mostly the foremost source of these markers.[16]

These protein markers like nucleic acids and proteins can be easily recognized in different body fluids like plasma/serum, urine, saliva, bronchoalveolar lavage fluid, cerebrospinal fluid and other body fluids which are secreted by the body where a solid tumor has been developing. These molecular markers or protein markers have been used for the early identification of the many leisons, and to make a decision for their therapeutic approach.[17,18]

DOES SALIVA AS BIOSPECIMEN FOR NON-INVASIVE AND ACCURATE DIAGNOSIS

Saliva has the compensation of easily accessible in a non-invasive manner, low background of normal material (cells, DNR, RNA and proteins) and inhibitory substances and less complex than blood.[4]

Blood contains more proteins than saliva, assaying trace amounts of factors may result in a greater risk of non specific interference and a greater chance for hydrostatic (and other) interactions between the factors and the abundant blood proteins. Blood also possess numerous carrier proteins such as albumin that must either be removed or treated prior to be assayed for protein content.

Also due to blood's direct connection with multiple organs of the body, the search for the cancer- related biomarkers may be clouded by the comorbidity effects of one disease influencing the protein profile of another.[19]

The Whole saliva is the product of the secretions of the 3 major salivary glands (parotid, submandibular, sublingual) and the numerous minor salivary glands mixed with crevicular fluid, bronchial and nasal secretions, blood constituents from wounds or bleeding gum, bacteria, viruses, fungi, exfoliated epithelial cells and food debris.[20]

Whole saliva can be collected with or without stimulation. Stimulation can be performed with masticatory movements or by gustatory stimulation (citric acid). Unstimulated saliva can be collected by merely spitting in a test tube or by leaving saliva drool from the lower lip and it is more often used for the diagnosis or follow up of systemic diseases.[21]

BIO-MARKERS

The United Nations' World Health Organization defines a biomarker as any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease.[22]

CRITERION FOR BIOMARKER[23]

1. A stable product, not vulnerable to artefactual induction, not easy to lose, or not changeable during storage.
2. Determined by an analytical assay that is specific, sensitive, reproducible and robust
3. A major product of oxidative modification that may be implicated directly in the development of the disease
4. Representative of the balance between oxidative damage generation and clearance
5. Accessible in a target tissue or a valid surrogate tissue such as a leukocyte
6. Free of confounding and interference factors from dietary intake
7. Detectable and measurable within the limits of detection of a reliable analytical procedure.

SALIVARY BIOMARKERS

Different salivary biomarkers are now being identified using state-of-the-art genomic or proteomic techniques which can be used for diagnostic purpose (Table 1).[24]

Table 1

Salivary Biomarkers with Their Possibilities for Use

Saliva/Oral Fluid Biomarkers	Possibilities for Use
DNA	Standard genotyping
	Bacterial infection
	Diagnosing carcinomas of the head and neck
	Forensics
RNA	Viral/bacterial identification
	Carcinomas of the head and neck
Proteins	Diagnosing periodontitis
	Diagnosing carcinomas of the head and neck
	Detecting dental cavities
Mucins/glycoproteins	Diagnosing carcinomas of the head and neck
	Detecting dental cavities
Immunoglobulins	Diagnosing viruses (HIV, hepatitis B and C)
Metabolites	Diagnosing periodontitis
Drugs and their metabolites	monitoring drug abuse
	Detecting of drugs in the body
Viruses, bacteria	Epstein-Barr virus reactivation (mononucleosis)

Cellular material diagnosing carcinomas of the head and neck.

There has been speculations about possible mechanisms that lead to the presence of tumor markers in the saliva.

These markers can be:

- may be derived from serum or

- locally produced

Serum derived nucleic acids and proteins in the saliva may be part of the normal salivary secretion (by the acinar cells).[25] Biomolecules from serum may enter saliva by either passive diffusion, active transport via ligand-receptor binding, or ultrafiltration[26] or as constituents of the outflowing crevicular fluid.

The most common route for substances to migrate from blood to saliva is via unaided or passive diffusion.[27]

Cell necrosis, lysis or apoptosis and trauma of the epithelial cell or cancerous cells lead to the local production of the tumor markers. These markers may even be actively released by normal epithelial or cancerous cells which may further contribute to the local production.

There is mounting evidence exists concerning the presence of cell-free nucleic acids and proteins in apoptotic bodies. An important step in cell disposal is degradation and separation of these nucleic acids in the apoptotic bodies. In addition to DNA, tumor-specific RNA has been also found recently in serum and plasma samples from cancer patients. In a study done by *Halika et al*. (2000), they found that RNA was present in a single or in two distinct and relatively large apoptotic bodies which also protect these molecules from degradation.[28-29]

Circular membrane fragments called microvesicles (MV) are sheded from surface membranes as well as secreted from the endosomal membrane compartment of normal and malignant cells.

MV contains numerous proteins and lipids similar to those present in the membranes of the cells from which they originate. Furthermore, as MV membranes engulf some cytoplasm during membrane blebbing, they may also contain proteins derived from it and mRNA.[30]

Molecular markers for the diagnosis of OSCC can be quested in 3 levels;
(I) changes in the cellular DNA, (which result in)
(II) altered mRNA transcripts, (leading to)
(III) altered protein levels (intracellularly, on the cell surface or extracellularly).[17]

SALIVARY GENOMICS

a) Cellular DNA

The tumor-specific DNA in saliva could be used as a biomarker of OSCC.[3]

Salivary genetic and epigenetic analysis reflects pathological genetic processes such as aberrant gene transcription profiles for example, cancers.

The salivary genome is made up of DNAs representing the genome of the individual, oral microbiota and infecting DNA viruses. Compared to blood and urine, the quantity and quality of DNA that can be obtained from saliva is relatively good and can be used for genotyping, amplifi- cation or sequencing and can be stored long-term without significant degradation Salivary DNA is a robust analyte reflecting presence or absence of specific genes, alteration to sequences (mutation) and methylation status but limitation is that it cannot provide information on upregulation and down- regulation of gene expression.[32]

Changes seen in the host DNA of dysplastic or cancer cells are due to point mutations, deletions, translocations, amplifications and methylations, cyclin D1, epidermal growth factor receptor, microsatellite instability and human papillomavirus presence.[17]

The first report of saliva as a diagnostic tool for oral cancer detection was published in 2000 by *Liao et al*. They claimed that exon 4, codon 63 of the p53 gene was mutated in salivary DNA from five of eight (62.5%) oral cancers patients.[33]

Aberrant methylation of tumor suppressor genes is common in cancer cells, and in oral squamous cell carcinoma (OSCC) hypermethylation has been linked with several cancer-related alterations of dysplastic oral epithelium.[34]

Promoter hypermethylation has been reported in premalignant OSCC lesions and in head and neck squamous cell carcinoma (HNSCC), showing potential as a biomarker for early detection of primary and relapsing OSCC or HNSCC.[32] Allelic loss on chromosomes 9p has also been noted in OSCC.[17]

Shiptzer et al. (2009) reported increased salivary levels of cell cycle regulatory proteins including Cyclin D1 and ki67, glycolytic enzyme lactate dehydrogenase (LDH), matrix metalloproteinase (MMP)-9, as well as reduction in DNA repair enzyme, 8-oxoquanine DNA glycosylase (OGG1) and Maspin, a tumor suppressor protein in oral cancer patients.[35]

Microorganism infection involved in OSCC development was confirmed by *Mager et al.* They used checkerboard DNA–DNA hybridization to conclud that oral cancer subjects had elevated counts of C. gingivalis, P. melaninogenica and S. mitis in saliva compared to OSCC-free subjects.[2]

Recent research has been directed towards detecting the human papilloma virus (HPV) in saliva, as one of the etiological factor in oral cancer. The incidence of HPV positivity in patients treated for oral cancer is estimated to be more than 45%.[36]

In addition, DNA polymorphism of IL-1b, IL-6, IL-8, TNFa and VEGF were found to be associated with the development of OSCC. P53 VEGF and TNF-α were

correlated to the environmental carcinogen such as tobacco or areca (betel) chewing induced OSCC.[4]

b) RNA

mRNAs and miRNAs are secreted from cells into the extracellular milieu and can be found in biofluids that are distant to the cellular sources . In a diseased state, transcription of specific mRNAs and miRNAs is altered. Although the validity of salivary RNAs for the development of biomarkers with diagnostic potential initially received some criticism[40], this approach is now widely accepted. Microarray technology has allowed high-throughput saliva analysis and is the current gold standard for identifying saliva transcripts.[32]

i) mRNA

mRNA is the direct precursor of protein and as the corresponding levels are correlated in cells and tissue samples, hence salivary mRNA for dual specificity phosphatase 1 (DUSP1), H3 histone, family 3A (H3F3A), IL-1B, IL-8, ornithine decarboxylase antizyme 1 (OAZ1), spermidine/ spermine N1-acetyltransferase (SAT) and S100 calcium binding protein P (S100P) are documented in literature as the biomarkers of oral cancer.[36]

Four transcriptome [IL-8, IL-1B, spermidine/ spermine N1-acetyltransferase 1 (SAT1) and S100P] and three protein (IL-1B, IL-8 and M2BP) biomarkers which were found to be significantly elevated in oral cancer.[37]

Various studies done on polymorphism of several genes like IL-6, IL-8, TNF-a, vascular endothelial growth factor (VEGF), cytochrome P4501A1 (CYP1A1), glutathione-S-transferase T1 (GSTT1) and glutathione-S-transferase M1 (GSTM1) have shown association with the development of oral cancer.[36]

ii) miRNA

miRNAs are short (19-25 nucleotides) transcripts of RNA which are mostly associated with post-transcriptional regulation by the RNA-induced silencing complex. They are found to play a role in cell growth, differentiation, apoptosis, pathogen-host interactions and stress responses and immune function, and are found in saliva. Salivary miRNA are harbored in exosomes, making it a stable biomolecule. In several cancer cell types, miRNAs are differentially expressed compared with normal cells, with observed differences that range from 10 to over a 100-fold.[23]

miRNA has been found to be more useful for characterizing solid tumor types than mRNA. miRNA has been shown to precisely discriminate poorly differentiated tumors, whereas mRNA profiling on the same samples produced highly inaccurate results.

Therefore, miRNA's are potentially very powerful in serving as biomarkers in cancer. If miRNA-based approaches reveal similar differential profiles using saliva samples, they may become very useful in salivary diagnostics.

Significantly reduced levels of miRNAs miR-125a and miR- 200a (known tumor suppressors) in the saliva of oral cancer patients compared with controls was reported by *Park et al*. Recently, it is also shown that salivary miR-31 (implicated in tumorigenesis) was appreciably superior in all stages of oral cancer, and that salivary miR-31 was more copious than blood miR-31, representing the oral tumor origin of this biomarker.[32]

SALIVARY PROTEINS A BIOMARKERS

Salivary protein markers for OSCC have been investigated in various studies and have shown relatively moderate sensitivity and specificity values relative to prognosis prediction.

Protein markers are differentiation antigens of corresponding normal tissue and characterize a certain stage of its maturation. They originate from live cells and show high tissue specificity. However, they may be detected in other pathologies as well.

New and sophisticated approaches, such as Luminex Multianalyte Profiling (xMAP) technology, shotgun proteomics, capillary reversed-phase liquid chromatography with quadruple time off light mass spectrometry and matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) has contributed significantly in recent years to the research in saliva for cancer diagnosis.

Different studies have shown that saliva contains proteins that may serve as biomarkers for OSCC, since 46 peptides/proteins were found at significantly different levels between the OSCC and control groups.

Carbonylation (indicative of oxidative damage to proteins) associated with cancer is cytotoxic which is irreversible and irreparable in nature. It is currently reported that a substantial increase in salivary carbonyls (246%) is seen in OSCC patients.

IL-6, IL-8 are post-inflammatory cytokines playing a prominent role in immune host defense responses to infection. These chemokines influence tissue remodeling and take part in the regulation of cell proliferation and differentiation. They are known to stimulate angiogenesis. They are essential mediators of cancer development and powerful activators of apoptotic and anti-apoptotic signaling cascade. Hence, IL-6 and IL-8 have been implicated in early detection of oral pre malignancies and OSCC.

Different metalloproteinases are also found to be significantly altered in OSCC. MMP-9 expression in stromal cells surrounding the invading front of metastasizing tumors are to found to be high. MMP-9 polymorphism was shown to have a strong association

with increased risk for developing OSCC. In a study done by *Shpitzer, et al*., they found a 39% increase in MMP-9 with a sensitivity of 100% and specificity of 79% in OSCC patients.

The cytoskeletal intermediate filaments known as "cytokeratins" are present in almost all normal and malignant epithelial cells. The degradation of these cytokeratin free filaments by activated protease into the blood is increased in the malignant epithelial cells. In OSCC, it has been found that levels of Cyfra 21-1 are increased in saliva.

When compared with healthy controls elevated levels of salivary Defensin 1 are found in OSCC patients by *Mizukawa, et al*.

Few other salivary biomarkers which are significantly altered in OSCC patients as compared with healthy controls are inhibitors of apoptosis (IAP), squamous cell carcinoma associated antigen (SCC-Ag), carcino-embryonic antigen (CEA), carcino-antigen (CA19-9), CA128, serum tumor marker (CA125), tissue polypeptide specific antigen (PPS), reactive nitrogen species (RNS), lactate dehydrogenase (LDH) and immunoglobulin G (IgG), s-Ig A, insulin growth factor (IGF).[17,23,38]

OXIDATIVE STRESS RELATED BIOMARKERS

Bahar et al. documented that in oral cancer patients, the salivary reactive nitrogen species were significantly higher, while all salivary antioxidants were significantly lower in the as compared to the controls. This increase may be the event leading to the consumption and reduction of salivary antioxidants resulting in the oxidative damage to DNA and proteins, and possibly leading to progression of oral cancer.[36]

POTENTIAL SALIVARY BIOMARKERS REPORTED FOR ORAL CANCER DETECTION (TABLE 2)[39]

Category

1. Non-organic compound Na, Ca, F, and Mg
2. Peptide
   Defensin-1
3. Proteins P53 autoantibody

α-amylase
IL-8
TNF-α
IL-1
IL-6
Basic fibroblast growth factor
Statherin
Cyfra 21.1
TPA
CA125 E
ndothelin-1
IL-1β
CD44
IGF-1
MMP-2, MMP-9
CD59
Catalase
Profilin
S100A9/MRP14
M2BP
CEA
Carcinoma associated antigen CA-50
Salivary carbonyls
Cyclin D1
Maspin
8-oxoguanine DNA glycosylase
OGG1
Phosphorylated-Src
Ki-67
Lactate dehydrogenase
Transferrin
Zinc finger protein 501 peptide
Hemopexin
Haptoglobin
Complement C3
Transthyretin
α1-antitrypsin

4. DNAs
   P53 gene codon 63

Loss of heterozygosity in the combination of markers D3S1234, D9S156 and D17S799.

Mitochondrial DNAs (cytochrome c oxidase I and cytochrome c oxidase II).

Hypermethylation of promoters in tumor suppressor genes: DAPK, DCC, MINT-31, TIMP-31, TIMP-3, p16, MGMT, CCNA1

Presence of HPV, EBV

5. mRNAs IL-8 IL-1β DUSP1 H3F3A OAZ1 S100P SAT (spermidine/SAT EST)

6. MicroRNAs miR-125a miR-200a miR-31

7. Long non-coding RNAs
   HOTAIR

8. Oxidative stress-related molecules RNS such as NO, NO2 and NO3

Peroxidase
GST
SOD
8-OHdG
Glutathione
MDA

BARRIERS TO SALIVARY DIAGNOSTICS

The first barrier to salivary cancer diagnostics is making the medical and research community aware of the potential of salivary tumor markers in cancer detection. Currently, this novel approach needs to demonstrate its utility among these cohorts of researchers. Until the first salivary diagnostics procedures are internationally approved, little interest will be cultivated in this field of endeavor.

Second, the cost of development is a serious issue and may produce a significant barrier to progression of any diagnostic test. This problem may be solved by cooperative agreements between government agencies, academia and the private sector. Furthermore, if mass production is envisioned, then cost-effective manufacturing methods must be developed.[19]

CONCLUSION

Currently, the use of saliva in the field of research is rapidly evolving and advancing due to the use of novel approaches including metabolomics, genomics, proteomics and bioinformatics. Due to its proximity to oral cavity and non-invasive collection procedure, salivary screening can be the best choice as primary screening test for oral cancer. Although at present no single tumor marker is helpful, a panel of biomarkers would be more appropriate for validating the presence or prognosis of disease.

OSCC can be diagnosed with high sensitivity and specificity by merely testing saliva samples from the subjects. This does not of course undermine the value of screening tests by visual examination neither the importance of the tissue biopsy which is still the 'gold standards' in OSCC diagnosis.

Lastly, since the present methods are not ready for immediate clinical use as diagnostic tools, much work is necessary and it can be envisaged that simple, fast, portable and cost-effective clinical diagnostic systems could be available in the near future.[16,38,39]

REFERENCES

1. Feller L., Lemmer, J. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment. Journal of Cancer Therapy. 2012; 3(4).
2. Mager D. L., Haffajee A. D., Devlin P. M., Norris C. M., Posner M. R., & Goodson J. M. The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects (2005). Journal of translational medicine; 3(1): 27.
3. Hu S., Arellano M., Boontheung P., Wang J., Zhou H., Jiang J.et al). Salivary proteomics for oral cancer biomarker discovery (2008). Clin Cancer Res 2008; 14: 6246-6252.
4. Wu J. Y., Yi C., Chung H. R., Wang D. J., Chang W. C., Lee S. Y. et al W. C. V. Potential biomarkers in saliva for oral squamous cell carcinoma. Oral oncology; 46(4): 226-231.
5. Righini CA, de Fraipont F, Timsit JF, et al. Tumorspecific methylation in saliva: a promising biomarker for early detection of head and neck cancer recurrence. Clin Cancer Res 2007; 13: 1179-85.
6. Fedele S. Diagnostic aids in the screening of oral cancer. Head Neck Oncol 2009; 30: 1-5.
7. Zakzerwska JM, Hindle I, Speight PM. Practical considerations for the establishment of an oral cancer screening programme. Commun Dent Health 1993; 10(Suppl 1): 79-85.
8. Onofre MA, Sposto MR, Navarro CM. Reliability of toluidine application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2001; 91: 535-40.
9. Zhang L, Williams M, Poh CF, et al. Toluidine blue staining identifies high-risk primary oral premalignant lesions with poor outcome. Cancer Res., 2005; 65: 8017-21.
10. Christian DC. Computer-assisted analysis of oral brush biopsies at an oral cancer screening program. J Am Dent Assoc, 2002; 133: 357-62.
11. Mehrotra R, Hullmann M, Smeets R, Reichert TE, Driemel O. Oral cytology revisited. J Oral Pathol Med 2009; 38: 161-6.
12. Kerr AR, Sirois DA, Epstein JB. Clinical evaluation of chemiluminescent lighting:an adjunct for oral mucosal examinations. J Clin Dent., 2006; 17: 59-63.
13. Epstein JB, Silverman S, Jr, Epstein JD, Lonky SA, Bride MA. Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and tolouidine blue. Oral Oncol, 2008; 44: 538-44.
14. Onizawa K, Saginoya H, Furuya Y, Yoshida H. Fluorescence photography as a diagnostic method for oral cancer. Cancer Lett, 1996; 108: 61-6.
15. Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002; 2: 210 –9.
16. Li Y., John M. A. S., Zhou X., Kim Y., Sinha U., Jordan R., Wong, D. T. Salivary transcriptome diagnostics for oral cancer detection(2004). Clinical Cancer Research; 10(24): 8442-8450.
17. Markopoulos, A. K., Michailidou, E. Z., & Tzimagiorgis, G. Salivary markers for oral cancer detection. The open dentistry journal. 2010; 4: 172-7.
18. Sashikumar R, Kannan R. Salivary glucose levels and oral candidal carriage in type II diabetics. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 109(5): 706-11.
19. Streckfus, Charles F., and William P. Dubinsky. Proteomic analysis of saliva for cancer diagnosis. 2007; 329-332.
20. Chiappina S, Antonellia G, Gattib R, De Paloa E F. Saliva specimen: a new lab tool for diagnostic and

basic investigation. Clinica Chimica Acta, 2007; 383: 30–40.
21. Navazesh M. Methods for collecting saliva. Ann NY Acad Sci. 1993; 8: 72-7.
22. Mishra A, Verma M. Cancer biomarkers: are we ready for the prime time? Cancers (Basel), 2010; 2: 190-208.
23. Bano S, David MP, Indira AP. Salivary biomarkers for oral squamous cell carcinoma: An overview. IJSS Case Reports & Reviews, 2015; 1(8): 39-45.
24. Sandhu SV, Bhandari R, Gupta S, Puri A. Salivary Diagnostics: An Insight. Indian Journal of Dental Sciences. 2011 Dec 1; 3(5).
25. Baum BJ. Principles of saliva secretion. Ann NY Acad Sci., 1993; 694: 17-23.
26. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: Current state and future applications. Clinical Chemistry. 2011; 57(5): 675–687.
27. Haeckel R, Hanecke P. Application of saliva for drug monitoring: an in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem. 1996; 34: 171–91.
28. Halicka HD, Bedner E, Darzynkiewicz Z. Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. Exp Cell Res., 2000; 260: 248-56.
29. Hasselmann D, Rappl G, Tilgen W, Reinhold U. Extracellular tyrosinase mRNA within apoptotic bodies is protected from degradation in human serum. Clin Chem 2001; 47: 1488-9.
30. Baj-Krzyworzeka M, Szatanek R, Weglarczyk K, Baran J, Urbanowicz B, Branski P et al. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. Cancer Immunol Immunother 2006; 55: 808–818.
31. Zimmermann BG, Wong DT. Salivary mRNA targets for cancer diagnostics. Oral Oncol, 2008; 44(5): 425–9.
32. Bonne NJ, Wong DTW: Salivary biomarker development using genomic, proteomic and metabolomic approaches. Genome Medicine, 2012; 4: 82.
33. Liao PH, Chang YC, Huang MF, Tai KW, Chou MY. Mutation of p53 gene codon 63 in saliva as a molecular marker for oral squamous cell carcinomas. Oral Oncol, 2000; 36(3): 272–6.
34. Shaw R: The epigenetics of oral cancer. Int J Oral Maxillofac Surg, 2006; 35: 101-108.
35. Shiptzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, et al. Salivary analysis of oral cancer biomarkers. Br J Cancer. 2009; 101: 1194–8.
36. Shah, Franky D., et al. "A review on salivary genomics and proteomics biomarkers in oral cancer." Indian Journal of Clinical Biochemistry, 2011; 26.4: 326-334.
37. Brinkmann O, Kastratovic DA, Dimitrijevic MV, Konstantinovic VS, Jelovac DB, Antic J, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. Oral Oncol. 2011; 47: 51–5.
38. Krishna Prasad RB, Sharma A, Babu HM. An insight into salivary markers in oral cancer. Dent Res J (Isfahan) 2013; 10: 287-95.
39. Cheng YS, Rees T, Wright J. A review of research on salivary biomarkers for oral cancer detection. Clin Transl Med, 2014; 3: 3.