Protein Biomarkers in Saliva in Oral Squamous Cell Carcinoma.

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Abstract

Diagnosis of oral squamous cell carcinoma (OSCC) relies mainly on the histopathological analysis of the biopsied tissue which is always traumatic for the patient. Salivary biomarkers might be an important adjunct to the diagnosis of OSCC especially in early stages of the disease because of the ease and non-invasiveness of specimen collection. Many salivary biomarkers have already been introduced. This article is a review of the protein biomarkers that have been investigated for their usefulness in association to OSCC.

Key Words: Oral cancer, biomarker, protein, salivary diagnostics

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Introduction

Oral cancer is ranked as the eleventh most common cancer around the world [1]. Oral cancer is the generalized term for cancers that arise from lip mucosa anteriorly to the pharyngeal mucosa posteriorly [2]. Oral squamous cell carcinoma is the most frequently encountered carcinoma that accounts for greater than 90% of all malignant oral cancers [3]. Patients with OSCC usually seek medical attention only when the disease has already progressed to the extent that it presents with chronic non-healing ulcer or recurrent bleeding or unusually large exophytic mass. The five-year survival rate of OSCC remains low, between 15 to 50 % when a diagnosis is made at the late stages of the disease, but rises considerably up to 85% when an early diagnosis is made [4]. The delay in diagnosis remains the key factor for increased mortality in OSSC.

Histopathological examination of tissue obtained after biopsy remains the gold standard for the diagnosis of OSCC. Performing biopsy to achieve the precise tissue that represents the lesion may sometimes be difficult especially in non-uniform exophytic masses with superinfection or when a lesion presents with a frank bleeding. Incisional biopsy is the biopsy of choice for histopathological examination of OSCC which is invasive hence traumatic for the patients in all instances. To overcome this hurdle, the concept of diagnosing OSCC from sources other than biopsied tissue is already in progress. These diagnostic sources typically include body fluids especially serum and saliva of patients [5, 6].

Saliva, which is often regarded as a reflection of human health, is enriched with various types of inorganic and organic molecules. The organic components of saliva constitute of endogenously secreted proteins such as IgA, salivary enzymes such as amylase, lingual lipase, and lysozyme. There are many hormones including insulin, steroids such as cortisol, testosterone, and progesterone as well as various peptides including metalloprotease, leptin, and lactoferrin which are secreted in saliva by passive diffusion [7, 8]. Thus, saliva serves as an excellent source for assessing not only the oral health but also the overall systemic health of an individual by monitoring the endogenous and exogenous compounds present in it [8] .

The molecular events of carcinogenesis begin much earlier and before the clinical presentation of the disease is evident. Carcinogenesis involves a deregulation of numerous types of molecules starting from DNAs to the breakdown of extracellular matrix proteins such as collagen as well as increased expression of molecules that promote tumor cell proliferation, invasion and angiogenesis. The downregulated and upregulated molecules as well as fragments of intracellular components including DNAs, RNAs or small polypeptides can enter saliva via the passive diffusion pathway. Likewise, exfoliated tumor cells from oral cavity are most likely to be found floating in the saliva. Saliva is therefore proposed to be an important source of biomarkers for the diagnosis, monitoring the progression, determining the disease outcome, and predicting the prognosis of OSCC [7, 9, 10].

The National Cancer Institute of USA defines biomarker as “A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a

treatment for disease or condition. Also called molecular marker and signature molecule." The definition holds true for conditions like cancer. Thus, biomarkers can be any molecules such as DNAs, RNAs, peptides, enzymes or any deregulated molecular events during carcinogenesis [11]. Biomarkers for oral cancer can be searched at 3 basic levels: alterations in DNA which result into alteration in the RNA transcripts finally leading to changes in the protein translates. Accordingly, salivary genomics, transcriptomics and proteomics are the major areas where biomarkers can be quested. As far as salivary proteomics is concerned, proteins are mainly synthesized by the salivary acinar cells and consequently secreted with saliva into the oral cavity. A few amount of proteins secreted in saliva might have been through the process of passive diffusion [9, 12]. Therefore, salivary proteomics can be useful for recognizing biomarkers for native as well as distant diseases. Table 1 shows the protein biomarkers in saliva that have been studied from over the past few decades till date for OSCC.

Table 1. Protein biomarkers in saliva for OSCC detection, reported as of 2016.

Group	Protein markers in saliva for OSCC	References	Technique for protein quantification and analysis
Cytokines	Interleukin-1α (IL-1α)	Rhodus et al. 2005 [13]	Enzyme Linked Immunosorbent Assay [ELISA]
	IL-1β	Katakura et al. 2007 [14]	ELISA
		Brinkmann et al. 2011 [15]	ELISA
		Elashoff et al. 2012 [16]	ELISA
		Kamatani et al. 2013 [17]	Suspension Array System [SAT]
	IL-6	Rhodus et al. 2005 [13]	ELISA
		Katakura et al. 2007 [14]	ELISA
		SaheebJamee et al. 2008 [18]	ELISA
		Sato et al. 2010 (19)	Chemiluminescent Enzyme Immunoassay [CL-EIA]
	IL-8	St. John et al. 2005 [20]	ELISA
		Rhodus et al. 2005 [13]	ELISA
		Brinkmann et al. 2011 [15]	ELISA
		Elashoff et al. 2012 [16]	ELISA
		Punyani and Sathawanee. 2013 [8]	ELISA
	IL-10	Aziz et al. 2015 [21]	Multianalyte Profiling [MAP]
		Malgorzata et al. 2016 [22]	ELISA

Group	Protein markers in saliva for OSCC	References	Technique for protein quantification and analysis
	IL-13	Aziz et al. 2015 [21]	Multianalyte Profiling [MAP]
	Tumor Necrosis Factor- α [TNF-α]	Rhodus et al. 2005 [13] Malgorzata et al. 2016 [22]	ELISA ELISA
Growth Factors	Transforming Growth Factor-β [TGF-β]	Malgorzata et al. 2016 [22]	ELISA
	Vascular Endothelial Growth Factor [VEGF]	Upile et al. 2009 [23] Andisheh-Tadbir et al. 2014 [24] Malgorzata et al. 2016 [22]	Solid phase ELISA ELISA ELISA
	Epidermal Growth Factor [EGF]	Shpitzer et al. 2007 [25] Bernardes et al. 2010 [26] Farzaneh Agha-Hosseini et. al. 2015 [27]	Solid phase ELISA ELISA ELISA
	Insulin-like Growth Factor-1 [IGF-1]	Shpitzer et al. 2007 [25]	Solid phase ELISA
	Fibroblast Growth Factor	Vucicevic et al. 2005 [28] Gorugantula et al. 2012 [29]	ELISA
Enzymes	α- amylase	Bassalyk et al. 1992 [30] Chen et al. 2002 [31]	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [MALDI-TOF MS]
	Catalase	Hu et al. 2008 [32]	2 dimensional gel electrophoresis [2 DE] and reverse-phase liquid chromatography [LC], followed by LC-tandem mass spectrometry [MS]
	Lactate Dehydrogenase [LDH]	Shpitzer et al. 2009 [33] Shetty et al. 2012 [34] Patel & Metgud. 2015 [35]	Kinetic Spectrophotometry Spectrophotometry Calorimetric Assay
	Telomerase activity	Zhong et al. 2005 [36]	PCR-ELISA followed by colorimetric assay
Oxidative-Stress Related Molecules	Nitric oxide, Nitrates, Nitrites	Bahar et al. 2007 [37]	Nitric Oxide and the Total Nitric Oxide Assays

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	Peroxidase	Bahar et al. 2007 [37]	Calorimetric Assaay
	Glutathione-S-Transferase	Bahar et al. 2007 [37]	Enzyme-Immunoassay [EIA]
	Superoxide dismutase	Bahar et al. 2007 [37]	Spectrophotometric Assay
	8-hydroxy-2 deoxyguanosine [8-OHdG]	Agha Hosseini et al. 2012 [38] Kaur et al. 2016 [39]	ELISA ELISA
	Malondialdehyde [MDA]	Agha Hosseini et al. 2012 [38] Kaur et al. 2016 [39]	Thiobarbituric acid [TBA] Thiobarbituric acid [TBA]
	Glutathione	Almadori et al. 2007 [40]	High Performance Liquid Chromatography [HPLC]
	Salivary Carbonyls	Bahar et al. 2007 [37] Shpitzer et al. 2009 [33]	Western blot using specific anti dinitrophenyhydrazine [DNPH] followed by sodium dodecyl sulfate polyacralamide gel electrophoresis [SDS-PAGE] ELISA followed by colorimetric test
	8-oxoguanine DNA glycosylase [OGG1]	Shpitzer et al. 2009 [33]	ELISA followed by colorimetric test
Plasma Proteins	Transferrin	Jou et al. 2010 [41]	2DE followed by MALDI-TOF MS and confirmed by MALDI TOF/TOF MS, Western blotting and ELISA
	Hemopexin Haptoglobin Transthyretin	Jessie et al. 2013 [42]	2DE followed by MS and validated by ELISA
Cytokeratins	Tissue Polypeptide Antigen [TPA]	Nagler et al. 2006 [43]	Immunoradiometric assay [IRA]
	Cyfra 21-1	Nagler et al. 2006 [43] Rajkumar et al. 2015 [44] Malhotra et al. 2016 [45]	Immunoradiometric assay [IRA] ELISA Electro-chemiluminescent Analysis

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Serine Protease Inhibitors	Maspin	Shpitzer et al. 2009 [33]	ELISA
	α1- antitrypsin	Jessie et al. 2013 [42]	2DE followed by MS and validated by ELISA
Complement Proteins	Complement protein C3	Jessie et al. 2013 [42]	2DE followed by MS and validated by ELISA
	Complement Activation Product C4d	Ajona et al. 2015 [46]	ELISA
	Complement Regulatory Glycoprotein CD59	Hu et al. 2008 [32]	2 DE and reverse-phase LC, followed by LC-tandem MS
Matrix Metalloproteinases [MMP]	MMP-2 MMP-9	Shpitzer et al. 2007 [25]	Solid phase ELISA
	MMP-9	Shpitzer et al. 2009 [33]	ELISA
	MMP-1 MMP-3	Stott-Miller et al. 2011 [47]	multiplex sandwich-ELISA proteome array
Cancer antigens	Cancer Antigen 125 [CA125]	Nagler et al. 2006 [43] Balan et al. 2012 [48]	ELISA ELISA
	Carcinoembryogenic antigen [CEA]	Nagler et al. 2006 [43] He et al. 2009 [49] Honarmand et al. 2016 [50]	ELISA ELISA ELISA
	Carcinoma-associated antigen 50 [CA-50]	He et al. 2009 [49]	ELISA
	Cancer antigen 19-9	Nagler et al. 2006 [43]	ELISA
Other Proteins	Defensin	Mizukawa et al. 1998 [51]	Reversed-phase HPLC followed by MS
	Statherin	Contucci et al. 2005 [52]	HPLC
	P53 autoantibody	Warnakulasuriya et al. 2000 [53]	ELISA followed by SDS-PAGE
	Endothelin-1	Pickering et al. 2007 [54] Cheng et al. 2011 [55] Hoffmann et al. 2011 [56]	ELISA ELISA ELISA
	CD44	Franzmann et al. 2007 [57]	ELISA

Group	Protein markers in saliva for OSCC	References	Technique for protein quantification and analysis
	Mac-2 Binding Protein [M2BP]	Hu et al. 2008 [32]	2 DE and reverse-phase LC, followed by LC-tandem MS
		Brinkmann et al. 2011 [15]	ELISA
		Elashoff et al. 2012 [16]	ELISA
	Cyclin D1 Phosphorylated Src Ki-67	Shpitzer et al. 2009 [33]	ELISA
	Profilin S100A9	Hu et al. 2008 [32]	2 DE and reverse-phase LC, followed by LC-tandem MS
	Zinc Finger Protein 510 [ZnF510]	Jou et al. 2011 [58]	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [MALDI-TOF MS]
	Salivary Total Protein	Shpitzer et al. 2007 [25] Shivashankara et al. 2011 [59]	Lowry method

Discussion

The use of salivary proteomics for the diagnosis of OSCC is promising. However there are certain limitations in OSCC salivary proteomics research. There are various techniques available for protein identification and quantification. Some of them such as SDS-PAGE, MALDI-TOF MS, HPLC or even PCR are too much elaborated and sophisticated that a routine diagnosis using these equipments becomes impractical in clinical setting in terms of the time and the expense.

There is no standard protocol of saliva collection, processing, and storage. Many researchers have preferred to collect the morning at least 1 hour after food intake [14, 20, 37, 43]. However, Rhodus et al. and Agha Hosseini et al. collected salivary samples 1 and a half hour after food intake [13, 27]. The rate and force of centrifugation for saliva processing also varies greatly from one study to the others, for example for 15 minutes at 2,600 g [15, 20, 55] for 10 minutes at 800 g [33, 37, 43]; for 20 minutes at 14,000 g. [42]; for 20 minutes at 14,000 rpm [54] or ; for 15 minutes at 3,500 rpm [14]. In many studies, saliva was stored at -80°C; however in studies by Mizukawa et al., Agha-Hosseini et al., and Shetty et al., saliva storage was done at -20°C [34, 38, 51]; at -70°C in the study by SahebJamee et al. [18]; at 4°C in a study by Almadori et al. [40]; and at 0°C by Katakura et al. [14] However, these studies have not clarified the time up to which the saliva samples can be stored before initiating the thawing process. Protease inhibitors were added in saliva before storage in some studies [14, 20, 29, 32, 55] while in others information about addition of such

inhibitors was not evident [8, 13, 25]. These variations in saliva handling from one study to the others raise a question that which saliva handling technique is the best one as far as reliability and reproducibility in different labs are concerned. A standard protocol in saliva handling must be made in order that salivary biomarkers could be used for detection of OSCC.

A wide variability has been observed in the levels of potential salivary biomarkers especially the cytokines in OSCC patients and even in apparently healthy individuals. Interleukins have shown the greatest levels of variability. Salivary IL-6 and IL-8 concentrations in OSCC patients were found to be 40.9±79.5 pg/ml and 1,093.7±1,089.0 pg/ml respectively in the study by SahebJamee et al. [18] whereas, Rhodus et al. found the salivary concentrations of IL-6 and IL-8 to be 88.2±43.2 pg/ml and 3,154.1±1,023.2 pg/ml in their study [13]. In controls, these studies showed salivary concentrations of IL-8 to be 700.7±1,031.5 pg/ml and 1,580±789 pg/ml respectively. From these two studies, an assumption can be made that there are certain other patient or control related confounding factors which led to such a wide variation in the levels of interleukins despite the fact that the assessment was done in healthy controls and OSCC patients in both studies. The difference in race, ethnicity, geography and general physiological and nutritional status of the study subjects as well as the difference in laboratory procedures might have resulted in such a variation.

The inflammatory cytokines such as interleukins as well as growth factors such as FGF, VEGF are raised during the process of inflammation as well as wound healing [60]. MMPs also show significantly higher levels in oral inflammatory conditions such as periodontitis [61]. Therefore, biomarker research should differentiate and validate potential OSCC salivary biomarkers in people having oral inflammatory diseases. Similarly, the biomarkers should have a very high specificity and sensitivity. If a biomarker shows high levels during inflammatory oral conditions to a point that there is no significant difference between that level and the levels observed in OSCC patients, its specificity falls drastically which ultimately undermines its use as a potential biomarker.

Many of the potential salivary OSCC biomarkers are also observed in other types of pathological conditions and tumors. For example LDH isoenzymes are frequently observed in body fluids in hepatic or cardiac insults [62]; endothelin-1 has been detected in patients suffering from heart failure [63] as well as other diseases such as gastritis, gastric- and duodenal ulcers [64]. Similarly, biomarkers such as CD44, profilin, CA 125, α-1 antitrypsin, maspin, p53 autoantibody are seen in body fluids such as serum or saliva in various tumors [65-70]. The presence of more than one tumor in the same patient may alter the levels of these biomarkers. Therefore, a determination of the specificity of potential OSCC biomarkers is necessary particularly in patients who have concomitant tumors in other parts of the body.

Conclusion

Although salivary biomarkers can serve as potential adjuncts tools for OSCC diagnosis, the limitations and variability associated with saliva collection, processing, storage as well as the issues regarding the reliability of approach to detect specific biomarkers and their sensitivity

and specificity in detecting OSCC have to be addressed before applying them for clinical use. This, however, paves the pathway for future studies and researches in this field.

References

1. World Health Organisation (WHO). Global Data on Incidence of Oral Cancer WHO/ NMH/CHP/HPR/ORH; 2005.

2. Sankaranarayanan R, Ramadas K, Amarasinghe H, Subramanian S, Johnson N. Oral cancer: prevention, early detection, and treatment. In: Gelband H, Jha P, Sankaranarayanan R, Horton S, eds. Cancer: disease control priorities, 3rd edn (Volume 3). Washington, DC: The International Bank for Reconstruction and Development/ The World Bank, 2015.

3. Chi AC. Epithelial Pathology. In: Brad W. Neville, Douglas D. Damm, Carl M. Allen, Bouquot JE. editors. Oral and Maxillofacial Pathology. 3rd ed. St. Louis: W.B. Saunders 2009. p409-421.

4. Prasad G, McCullough M. Chemokines and Cytokines as Salivary Biomarkers for the Early Diagnosis of Oral Cancer. Int J Dent. 2013; 2013: 1-7.

5. Mehrotra R, Gupta DK. Exciting new advances in oral cancer diagnosis: avenues to early detection. Head Neck Oncol 2011; 3(1): 1-9.

6. Guerra EN, Acevedo AC, Leite AF, Gozal D, Chardin H, De Luca Canto G. Diagnostic capability of salivary biomarkers in the assessment of head and neck cancer: A systematic review and meta-analysis. Oral Oncol 2015; 51(9): 805-818.

7. Lewis JG. Steroid Analysis in Saliva: An overview. Clin Biochem Rev. 2006; 27(3): 139-146.

8. Punyani SR, Sathawane RS. Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma. Clin Oral Investig. 2013; 17(2): 517-524.

9. Krishna Prasad RB, Sharma A, Babu HM. An insight into salivary markers in oral cancer. Dent Res J 2013; 10(3): 287-295.

10. Sinevici N, O'sullivan J. Oral cancer: Deregulated molecular events and their use as biomarkers. Oral Oncol 2016; 61: 12-18.

11. Malati T. Tumour markers: An overview. Indian J Clin Biochem 2007; 22(2): 17-31.

12. Yakob M, Fuentes L, Wang MB, Abemayor E, Wong DTW. Salivary Biomarkers for Detection of Oral Squamous Cell Carcinoma: Current State and Recent Advances. Curr Oral Health Rep 2014; 1(2): 133-141.

13. 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): 42-45.

14. Katakura A, Kamiyama I, Takano N, Shibahara T, Muramatsu T, Ishihara K, et al. Comparison of salivary cytokine levels in oral cancer patients and healthy subjects. Bull Tokyo Dent Coll 2007; 48(4): 199-203.

15. 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(1): 51-55.

16. Elashoff D, Zhou H, Reiss J, Wang J, Xiao H, Henson B, et al. Prevalidation of salivary biomarkers for oral cancer detection. Cancer Epidemiol Biomarkers Prev. 2012; 21(4): 664-672.

17. Kamatani T, Shiogama S, Yoshihama Y, Kondo S, Shirota T, Shintani S. Interleukin-1 beta in unstimulated whole saliva is a potential biomarker for oral squamous cell carcinoma. Cytokine 2013; 64(2): 497-502.

18. SahebJamee M, Eslami M, Atarbashi-Moghadam F, Sarafnejad A. Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2008; 13(5): 292-295.

19. Sato J, Goto J, Murata T, Kitamori S, Yamazaki Y, Satoh A, et al. Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010; 110(3): 330-336.

20. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2004; 130(8): 929-935.

21. Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, Iqbal J, et al. Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest 2015; 33(7): 318-328.

22. Polz-Dacewicz M, Strycharz-Dudziak M, Dworzański J, Stec A, Kocot J. Salivary and serum IL-10, TNF-α, TGF-β, VEGF levels in oropharyngeal squamous cell carcinoma and correlation with HPV and EBV Infect Agent Cancer 2016; 11:45. doi: 10.1186/s13027-016-0093-6. eCollection 2016.

23. Upile T, Jerjes W, Kafas P, Harini S, Singh SU, Guyer M, et al. Salivary VEGF: a non-invasive angiogenic and lymphangiogenic proxy in head and neck cancer prognostication. Int Arch Med 2009; 2(1): 12.

24. Andisheh-Tadbir A, Hamzavi M, Rezvani G, Ashraf MJ, Fattahi MJ, Khademi B, et al. Tissue expression, serum and salivary levels of vascular endothelial growth factor in patients with HNSCC. Braz J Otorhinolaryngol 2014; 80: 503-507.

25. Shpitzer T, Bahar G, Feinmesser R, Nagler RM. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol 2007; 133(9): 613-617.

26. Bernardes VF, Gleber-Netto FO, Sousa SF, Silva TA, Aguiar MCF. Clinical significance of EGFR, Her-2 and EGF in oral squamous cell carcinoma: a case control study. J Exp Clin Cancer Res 2010; 29(1): 40.

27.Agha-Hosseini F, Mohebbian M, Sarookani MR, Harirchi I, Mirzaii-Dizgah I. Comparative Evaluation of EGF in Oral Lichen Planus and Oral Squamous Cell Carcinoma. Acta Medica Iranica. 2015; 53(8): 471-475.

28. Vucicevic Boras V, Cikes N, Lukac J, Virag M, Cekic-Arambasin A. Salivary and serum interleukin 6 and basic fibroblast growth factor levels in patients with oral squamous cell carcinoma. Minerva Stomatol 2005; 54(10): 569-573.

29. Gorugantula LM, Rees T, Plemons J, Chen H-S, Cheng Y-SL. Salivary Basic Fibroblast Growth Factorin Patients with Oral Squamous Cell Carcinoma or Oral Lichen Planus. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012; 114(2): 215-222.

30. Bassalyk LS, Gus'kova NK, Pashintseva LP, Liubimova NV. Enzyme and isoenzyme activity in patients with malignant tumors of the oral mucosa. Vopr Onkol 1992; 38(3): 291-299.

31. Chen YC, Li TY, Tsai MF. Analysis of the saliva from patients with oral cancer by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2002; 16(5): 364-369.

32. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, et al. Salivary Proteomics for Oral Cancer Biomarker Discovery. Clin Cancer Res 2008; 14(19): 6246-6252.

33. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, et al. Salivary analysis of oral cancer biomarkers. Br J Cancer 2009; 101(7): 1194-1198.

34. Shetty SR, Chadha R, Babu S, Kumari S, Bhat S, Achalli S. Salivary lactate dehydrogenase levels in oral leukoplakia and oral squamous cell carcinoma: a biochemical and clinicopathological study. J Cancer Res Ther 2012; 8 Suppl 1: S123-125.

35. Patel S, Metgud R. Estimation of salivary lactate dehydrogenase in oral leukoplakia and oral squamous cell carcinoma: a biochemical study. J Cancer Res Ther 2015; 11(1):119-123.

36. Zhong LP, Chen GF, Xu ZF, Zhang X, Ping FY, Zhao SF. Detection of telomerase activity in saliva from oral squamous cell carcinoma patients. Int J Oral Maxillofac Surg 2005; 34(5): 566-570.

37. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients. Cancer 2007; 109(1): 54-59.

38. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med 2012; 41(10): 736-740.

39. Kaur J, Politis C, Jacobs R. Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitamin C, and vitamin E in oral pre-cancer and cancer: diagnostic value and free radical mechanism of action. Clin Oral Investig 2016; 20(2): 315-319.

40. Almadori G, Bussu F, Galli J, Limongelli A, Persichilli S, Zappacosta B, et al. Salivary glutathione and uric acid levels in patients with head and neck squamous cell carcinoma. Head Neck 2007; 29(7): 648-654.

41. Jou YJ, Lin CD, Lai CH, Chen CH, Kao JY, Chen SY, et al. Proteomic identification of salivary transferrin as a biomarker for early detection of oral cancer. Anal Chim Acta 2010; 681(1): 41-48.

42. Jessie K, Jayapalan JJ, Ong K-C, Abdul Rahim ZH, Zain RM, Wong K-T, et al. Aberrant proteins in the saliva of patients with oral squamous cell carcinoma. Electrophoresis 2013; 34(17): 2495-2502.

43. Nagler R, Bahar G, Shpitzer T, Feinmesser R. Concomitant Analysis of Salivary Tumor Markers—A New Diagnostic Tool for Oral Cancer. Clin Cancer Res 2006; 12(13): 3979-3984.

44. Rajkumar K, Ramya R, Nandhini G, Rajashree P, Ramesh Kumar A, Nirmala Anandan S. Salivary and serum level of CYFRA 21-1 in oral precancer and oral squamous cell carcinoma. Oral Dis 2015; 21(1): 90-96.

45. Malhotra R, Urs AB, Chakravarti A, Kumar S, Gupta VK, Mahajan B. Correlation of Cyfra 21-1 levels in saliva and serum with CK19 mRNA expression in oral squamous cell carcinoma. Tumour Biol 2016; 37(7): 9263-9271.

46. Ajona D, Pajares MJ, Chiara MD, Rodrigo JP, Jantus-Lewintre E, Camps C, et al. Complement activation product C4d in oral and oropharyngeal squamous cell carcinoma. Oral Dis 2015; 21(7): 899-904.

47. Stott-Miller M, Houck JR, Lohavanichbutr P, Mendez E, Upton MP, Futran ND, et al. Tumor and salivary matrix metalloproteinase levels are strong diagnostic markers of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2011; 20(12): 2628-2636.

48. Balan JJ, Rao RS, Premalatha BR, Patil S. Analysis of tumor marker CA 125 in saliva of normal and oral squamous cell carcinoma patients: a comparative study. J Contemp Dent Pract 2012; 13(5): 671-675.

49. He H, Chen G, Zhou L, Liu Y. A joint detection of CEA and CA-50 levels in saliva and serum of patients with tumors in oral region and salivary gland. J Cancer Res Clin Oncol 2009; 135(10): 1315-1321.

50. Honarmand MH, Farhad-Mollashahi L, Nakhaee A, Nehi M. Salivary Levels of ErbB2 and CEA in Oral Squamous Cell Carcinoma Patients. Asian Pac J Cancer Prev 2016; 17(S3): 77-80.

51. Mizukawa N, Sugiyama K, Fukunaga J, Ueno T, Mishima K, Takagi S, et al. Defensin-1, a peptide detected in the saliva of oral squamous cell carcinoma patients. Anticancer Res 1998; 18(6b): 4645-4649.

52. Contucci AM, Inzitari R, Agostino S, Vitali A, Fiorita A, Cabras T, et al. Statherin levels in saliva of patients with precancerous and cancerous lesions of the oral cavity: a preliminary report. Oral Dis 2005; 11(2): 95-99.

53. 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; 192(1): 52-57.

54. Pickering V, Jordan RCK, Schmidt BL. Elevated salivary endothelin levels in oral cancer patients A pilot study. Oral Oncol 2007; 43(1): 37-41.

55. Cheng YS, Rees T, Jordan L, Oxford L, O'Brien J, Chen HS, et al. Salivary endothelin-1 potential for detecting oral cancer in patients with oral lichen planus or oral cancer in remission. Oral Oncol 2011; 47(12): 1122-1126.

56. Hoffmann RR, Yurgel LS, Campos MM. Evaluation of salivary endothelin-1 levels in oral squamous cell carcinoma and oral leukoplakia. Regul Pept 2011; 166(1): 55-58.

57. Franzmann EJ, Reategui EP, Pedroso F, Pernas FG, Karakullukcu BM, Carraway KL, et al. Soluble CD44 Is a Potential Marker for the Early Detection of Head and Neck Cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(7): 1348-1355.

58. Jou YJ, Lin CD, Lai CH, Tang CH, Huang SH, Tsai MH, et al. Salivary zinc finger protein 510 peptide as a novel biomarker for detection of oral squamous cell carcinoma in early stages. Clin Chim Acta 2011; 412(15–16): 1357-1365.

59. Shivashankara AR, Kavya PM. Salivary Total Protein, Sialic Acid, Lipid Peroxidation and Glutathione in Oral Squamous Cell Carcinoma. Biomedical Research 2011; 22(3): 355-359.

60. Tissue Renewal and Repair: Regeneration, Healing, and Fibrosis. In: Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS, editors. Robbins and Cotran PATHOLOGIC BASIS OF DISEASE. 7th ed. Philadelphia: Elsevier Saunders; 2005. p87-118.

61. Makela M, Salo T, Uitto V-J, Larjava H. Matrix Metalloproteinases (MMP-2 and MMP-9) of the Oral Cavity: Cellular Origin and Relationship to Periodontal Status. J Dent Res 1994; 73(8): 1397-1406.

62. R.K. Murray, D.K. Granner, P.A. Mayes, V.W. Rodwell (Eds.), Harper's illustrated biochemistry. 26th ed. New York: McGraw-Hill; 2003.

63. Denver R, Tzanidis A, Martin P, Krum H. Salivary endothelin concentrations in the assessment of chronic heart failure. Lancet 2000; 355(9202): 468-469.

64. Lam HC, Lo GH, Lee JK, Lu CC, Chu CH, Sun CC, et al. Salivary immunoreactive endothelin in patients with upper gastrointestinal diseases. J Cardiovasc Pharmacol 2004; 44 (Suppl 1): 413-417.

65. Coumans JV, Gau D, Poljak A, Wasinger V, Roy P, Moens PD. Profilin-1 overexpression in MDA-MB-231 breast cancer cells is associated with alterations in proteomics biomarkers of cell proliferation, survival, and motility as revealed by global proteomics analyses. OMICS 2014; 18(12): 778-791.

66. Perez BH, Gipson IK. Focus on Molecules: Human mucin MUC16. Exp Eye Res 2008; 87(5): 400-401.

67. Sun Z, Yang P. Role of imbalance between neutrophil elastase and α1-antitrypsin in cancer development and progression. Lancet Oncol 2004; 5(3): 182-190.

68. Berardi R, Morgese F, Onofri A, Mazzanti P, Pistelli M, Ballatore Z, et al. Role of maspin in cancer. Clin Transl Med 2013; 2(1): 8.

69. Basakran NS. CD44 as a potential diagnostic tumor marker. Saudi Med J 2015; 36(3): 273-279.

70. Lubin R, Schlichtholz B, Teillaud JL, Garay E, Bussel A, Wild CP. p53 antibodies in patients with various types of cancer: assay, identification, and characterization. Clin Cancer Res 1995; 1(12): 1463-1469.

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