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Identification of saliva biomarkers as tools for detection of early-stage oral squamous cell carcinoma

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ABSTRACT

Cancer of the flat squamous cells of the oral cavity is called Oral Squamous Cancer Cell (OSCC). OSCC is responsible for 95% of all oral cancers. In high-risk countries like Pakistan, OSCC has one of the highest mortality rates amongst the general demographic. With most OSCC cases being identified in later stages, the 5-year survival rates have remained as low as 50%. However, with early diagnosis, OSCC patients have been shown to have a better chance of surviving. To that effect, identifying potential biomarkers to act as diagnostic indicators for OSCC can help in early diagnosis of the disease. An emerging field for alternative detection of cancer are non-invasive methods that use saliva as a fluid for liquid biopsies. Saliva comprises proteomes, microbiomes, metabolomes, transcriptomes, and genomes that can be used as potential tools for the early detection of cancer. "Salivaomics" has established itself as a promising area for diagnostic and prognostic purposes. In this review paper, we perform a meta-analysis and a systematic literature review to catalogue biomarkers found in saliva that could potentially be used in the early diagnosis of OSCC.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Head and Neck Cancer (HNC) comprises of at least 8-10% of all cancer.[1] Globally, Head and Neck Cancer account for more than 330,000 deaths and 650,000 cases every year.[2] In 2012, an estimated 4% of the cancer incidences in Europe were HNC.[2] HNC are defined by the region where they begin. According to the National Cancer Institute, the HNC includes oral cavity, pharynx, larynx, paranasal sinuses, nasal cavity and salivary glands. Most HNC are Head and Neck Squamous Cell Carcinoma (HNSCC) which is a type of cancer that arise from the mucosal surfaces of larynx, oropharynx (OPSCC) and the oral cavity and accounts for 90% of HNC.[3]

Worldwide, oral cancer is the fourth most common cancer among males and the eighth most common cancer among the female population.[1] Oral Squamous Cancer Cell (OSCC), which is a cancer of the flat squamous cells of the oral cavity, is responsible for 95% of all oral cancer globally.[1] Among European countries, France has the highest rate of OSCC with high rates also noted in countries like Hungary, Slovakia and Slovenia.[4] The Indian Subcontinent i.e. Pakistan, India and Bangladesh, has one of the highest rates of oral squamous cell carcinoma in the world. In these high-risk countries, OSCC is the most common cancer in men and the third most common cancer in women.[4]

According to the Globocan 2020 report,[5] in Pakistan, Lip and Oral Cavity accounts for 9.5% of all cancer cases with this being the leading cause for cancer in males and the second leading cause for females with second highest mortality rate of 9.1% in Pakistan.[5]

Almost two-thirds of OSCC cases are first diagnosed in the later stages of cancer (Stage 3 and Stage 4) with patients having a 5-year survival rate of less than 50%.[6,7] Overall, the five-year survival rate of OSCC has remained at 50% in the last 30 years.[8] On the contrary, OSCC diagnosed in Stage 1 and Stage 2 has a survival rate of 80% and 65% respectively.[8] This means that early diagnosis of OSCC can help in better treatment of OSCC patients.

Traditionally used methods for diagnosis of OSCC include a visual clinical examination followed by a biopsy and a histopathological examination.[9] However, there are some limitations in these conventional methods such as visual clinical inspection has a specificity of 31%,[8] and needle biopsies have a risk of causing infection to the patients as well as damage to structures adjacent to the cancer site.[10] Incisional biopsies increase the risk of metastasis and can cause other complications like hematoma at the site of the biopsy.[10,11]

An emerging alternative method for the detection of cancer are liquid biopsies, which refer to the examination of non-solid biological tissues through body fluids.[12] Body fluids can include excreted fluids like urine, secreted fluids like breast milk and saliva, or those obtained through a needle, like blood or cerebrospinal fluid. Apart from being considered less invasive and easier to collect, one of the biggest advantages of liquid biopsies is that they have the ability to provide clinicians with real-time results regarding the presence of a disease. Recent research has found the use of biomarkers present in these body fluids for the detection of cancer.[13]

According to the National Institutes of Health (NIH), Biomarkers Definitions Working Group, "*biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention*". The World Health Organization (WHO) defines biomarkers as "*almost any measurement reflecting an interaction between a biological system and a potential hazard which may be biological, chemical or physical"*.[14]

Among the body fluids, the use of saliva has previously been reported as a convenient body fluid for liquid biopsies. Saliva is non-invasive, easy to collect and store, and does not provide discomfort to the patients. Besides this, saliva has an additional advantage over other body fluids like blood as saliva does not clot which means that in configuration it does not change. These offer a huge advantage to investigate the use of saliva for clinical diagnostic purposes.

Saliva is an extracellular liquid secreted by the three major salivary glands (parotid, submandibular and sublingual) along with minor salivary glands located throughout the oral mucosa.[15,16] Saliva is slightly acidic with a pH of 6.6. It is mostly a water-based compound comprising 99% of water, 0.2% organic and inorganic substances, 0.3% proteins and numerous cellular elements.[15,17] Due to the close proximity of the salivary glands to blood vessels, salivary proteomic studies revealed that 20-30% of salivary proteins are overlapping with plasma proteomes.[17]

The composition of saliva includes biomarkers from the proteomes,[18] microbiomes,[19] metabolomes,[20] transcriptomes and genomes,[21] which are sometimes referred to as "SalivaOmics".[15] This study attempts to undertake an analysis of existing literature and identify potential salivary biomarkers which can be used in the early detection of OSCC.

MATERIALS & METHODS

Source of Data Collection

PubMed was thoroughly searched for relevant articles published in 2015-2020 related to saliva biomarkers of OSCC. The data was collected from PubMed in September, 2020.

Search Strategy

A total of two sets of keywords were used in the selected databases which returned a combined total of 30 articles.

The keywords "Salivaomics" and "Oral Cancer" and "Oral squamous cell carcinoma" were used which gave us a total of 17 results. After screening, 5 articles that related to saliva biomarkers and OSCC were selected.

A second set of keywords: "Salivaomics, Oral Cancer, Diagnosis" was used together in a latter search which gave us a total of 13 articles to screen. Of these 13 articles, 4 were selected. This gave us a total of nine articles to look at.

The next step involved reading the references mentioned in these articles and then looking at the selected cross-reference articles in the initial references. Altogether, 96 articles were studied for potential saliva biomarkers.

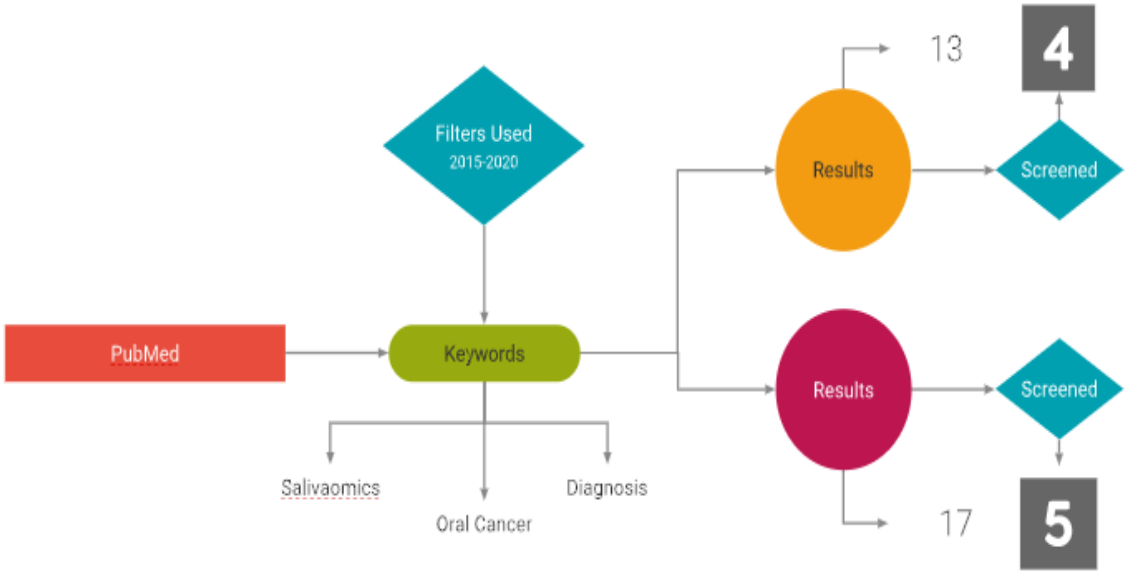

Figure 1: Summary of literature review from PubMed in September, 2020.

RESULTS

The salivary biomarker candidates were classified based on their type of source, i.e. genomics, proteomics, metabolomics, microbiomes and transcriptomics (Figure 2). They were then subcategorized into their respective molecule type (if any) like mRNA or microRNA in transcriptomics. Reported biomarkers in the saliva being involved in the development of OSCC were selected. A total of 129 salivary biomarkers were shortlisted. Of these the most investigated markers were of the transcriptomes, however, different proteomes were also studied in high numbers (Figure 2).

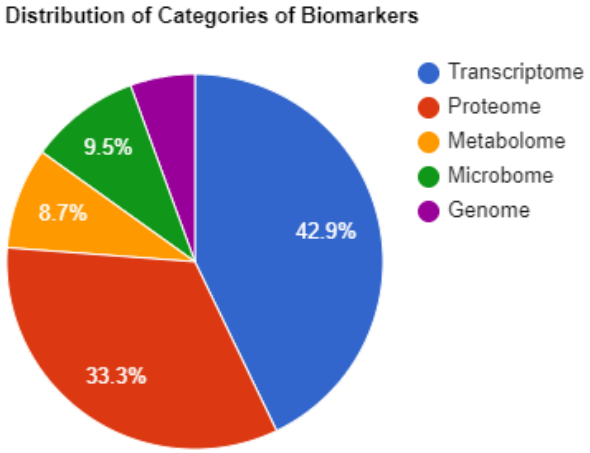

Figure 2: Distribution of OSCC saliva biomarkers reported showing biomarkers of transcriptome (in blue), proteome (in red), metabolome (in orange), microbiome (in green) and genome (in purple).

The distributions of the biomarkers are as follow: transcriptomes 42.9%, proteome 33.3%, metabolome 8.7%, microbiome 9.5%, genome 5.6% (Figure 2). Our results showed that among all the proteome biomarkers, interleukin-8 (IL-8) is the most reported biomarker followed by Interleukin-6 (IL-6), Tumour Necrosis Factor alpha (TNF-a) and Interleukin-1B (IL-1B) (Figure 3).

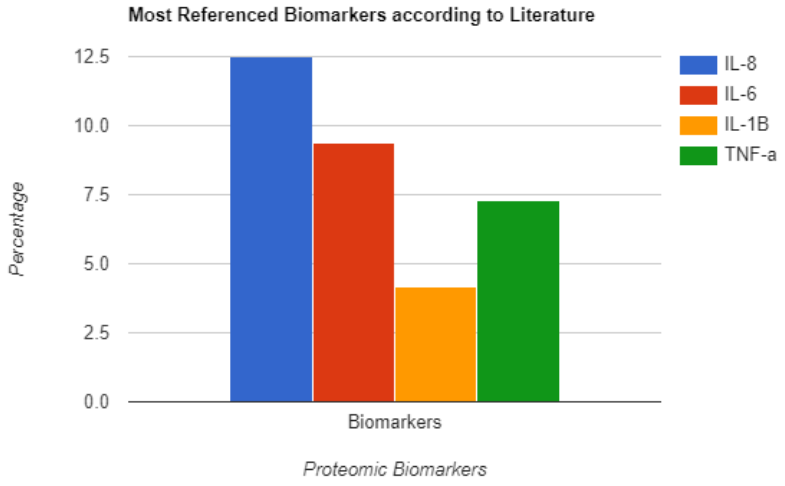

Figure 3: Most reported proteome biomarkers in the literature. Interleukin-8 (IL-8) (in blue) is the most reported biomarker followed by Interleukin-6 (IL-6) (in red), Tumour Necrosis Factor alpha (TNF-a) (in green) and Interleukin- 1B (IL-1B) (in yellow) respectively.

Among the transcriptomic saliva biomarkers, microRNAs were the most abundantly reported subcategory of saliva biomarkers having a potential for diagnosis of OSCC.

Therefore, these biomarkers were grouped together, and their diagnostic accuracies were evaluated according to the literature.

The evaluation was done on the basis of reported Area Under the Curve (AUC) of Receiver Operating Characteristic (ROC) in literature. A total of 45 miRNAs were compiled in which only 16 had AUC values. Of these 16 miRNAs, the top ten based on their reported AUC Values were selected as candidates for early-stage diagnosis. has-miR-136 had the highest AUC value of 0.9683 (Table 1).

Table 1: List of microRNA saliva biomarkers with reported AUC values in the literature.

microRNA Biomarker	AUC	References
has-miR-136	0.9683	(Momen-Heravi et al., 2014)[22]
has-miR-27b	0.9643	(Momen-Heravi et al., 2014)[22]
has-miR-375	0.957	(Harrandah et al., 2016)[23]
has-miR-412-3p	0.871	(Gai et al., 2018)[24]
has-miR-184	0.86	(Zahran et al., 2015)[25]
has-miR-512-3p	0.847	(Gai et al., 2018)[24]
has-miR-31	0.82	(Liu et al., 2010)[26]
has-miR-122-5p	0.73	(Salazar-Ruales et al., 2018)[27]
has-miR-21	0.73	(Zahran et al., 2015)[25]
has-miR-124-3p	0.71	(Salazar-Ruales et al., 2018)[27]

Some important messenger RNA (mRNAs) transcripts that have been reported in the literature were DUSP1, SAT1, OAZ1, S100P, H3F3A, transcript IL-8 and transcript IL-1β. The results showed that every study reported varying AUC values.

Table 2: List of mRNA biomarkers with AUC values reported in literature.

Biomarker	AUC Values (Li et al., 2004)[28]	AUC Values (Elashoff et al., 2012)[29]	AUC (Gleber-Netto et al., 2016)[30]	Sensitivity (Elashoff et al., 2012)[29]	Specificity (Elashoff et al., 2012)[29]	Sensitivity (Li et al., 2004)[28]	Specificity (Li et al., 2004)[28]
DUSP1	0.65	0.76	0.649	60%	56%	59%	75%
SAT1	0.7	0.8	0.643	66%	63%	81%	56%
OAZ1	0.69	0.73	0.519	62%	58%	100%	38%
S100P	0.71	0.78	0.597	60%	56%	72%	63%
H3F3A	0.68	0.74	0.524	61%	56%	53%	81%
IL-8	0.85	Nil	0.449	68%	64%	88%	81%
IL-1β	0.7	0.76	0.721	65%	60%	63%	72%

Our results show that top candidates for detection of OSCC at early stage includes IL-8, IL-6 proteins, has-miR-136, has-miR-27b, and has-miR-375 microRNA, DUSP1, SAT1, OAZ1, S100P, H3F3A, IL-8 and IL-1B from the mRNA biomarkers.

DISCUSSION

The potential of liquid biopsy in early diagnosis of cancer has been established now over the last decade. We found that in OSCC, early stage can be detected by using saliva biomarkers as a tool.

We found that the proteins of IL-8 and IL-6 had been reported to have increased in their expression in OSCC Patients about 3.5-fold as compared to healthy controls. [31,32]

Brinkmann et al., 2011[33] also supports this increase in expression of protein IL-8 in the saliva of OSCC patients in the Serbian population. Juretić et al., 2013[34] also found an increased concentration of IL-6 and TNF-a in the saliva of OSCC patients as well as in oral potentially malignant lesion.

However, according to Sahebjamee et al., 2008,[35] the concentration of IL-8 and TNF-a though was higher in OSCC patients, the difference was not statistically significant.

IL-1B has also been reported as an important diagnostic biomarker by Brinkmann et al., 2011,[33] according to which the concentration of this marker increases in OSCC patients. This is also in accordance with the studies published by Arellano-Garcia et al., 2008 and MAR St. John et al., 2004.[31,32]

According to Martin *et al.* 2015,[36] the concentration of DUSP1, SAT1, OAZ1, S100P are increased in OSCC patients. Brinkmann et al., 2011[33] has also reported the increased concentration of S100P, SAT 1 as well as transcript concentration of IL-8 and IL-B.

However, according to Gleber-Netto et al., 2016,[30] the concentration of DUSP1 in OSCC patients decreases as compared to healthy controls.

The miRNAs hsa-miR-124-3p has been reported by Salazar-Ruales et al., 2018[27] to have a decreased expression in OSCC patients. According to Harrandah et al., 2016[23] the concentration of hsa-miR-375 in OSCC patients is low. Momen-Heravi et al., 2014[22] has reported the downregulation of miRNA-136 in patients with OSCC. The miRNAs hsa-miR-412-3p, hsa-miR-512-3p have been validated by Rapado-González et al., 2019[37] and Gai et al., 2018.[24] They showed that these miRNAs have a higher concentration in OSCC patients. MicroRNAs hsa-miR-31, hsa-miR-184, hsa-miR-21, have been reported by Zahran et al., 2015[25] to have an increased concentration in OSCC patients. According to Momen-Heravi et al., 2014,[22] hsa-miR-27b has a higher expression in patients with OSCC. Salazar-Ruales et al.,

2018[27] has shown that hsa-miR-122-5p has a higher concentration in patients diagnosed with OSCC.

CONCLUSION

The detection of oral squamous cell carcinoma using saliva biomarkers must include a panel having multiple biomarkers from genome, transcriptome and proteome. However, experimental and clinical validation of these biomarkers is necessary in the Pakistani population.

REFERENCES

1. Ghalwash DM. Diagnostic and prognostic value of salivary biomarkers in oral cancer and precancer: review article. J Oral Maxillofac Surg Med Pathol. 2020;32(6):538-43. https://doi.org/10.1016/J.AJOMS.2020.06.013
2. Stenson KM. Epidemiology and risk factors for head and neck cancer. UpToDate. 2023 May 15. Available from: https://medilib.ir/uptodate/show/3390.
3. Vigneswaran N, Williams MD. Epidemiological trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am. 2014 May;26(2):123-41. https://doi.org/10.1016/J.COMS.2014.01.001.
4. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009 Apr-May;45(4-5):309-16. https://doi.org/10.1016/j.oraloncology.2008.06.002.
5. IARC. World Health Organization. Globocan. 2020. Pakistan. https://gco.iarc.fr/today/data/factsheets/populations/586-pakistan-fact-sheets.pdf.
6. Buch SA, Chatra L. Saliva as a non-invasive diagnostic medium in proteomics for oral squamous cell carcinoma detection. Int J Med Rev. 2019;6(3):73-6. https://doi.org/10.29252/IJMR-060301.
7. Speight PM, Epstein J, Kujan O, Lingen MW, Nagao T, Ranganathan K, et al. Screening for oral cancer—a perspective from the global oral cancer forum. Oral Surg Oral Medicine Oral Pathol Oral Radiol. 2017;123(6):680-87. https://doi.org/10.1016/J.OOOO.2016.08.021
8. Carreras-Torras C, Gay-Escoda C. Techniques for early diagnosis of oral squamous cell carcinoma: systematic review. Med Oral Patol Oral Cir Bucal. 2015 May 1;20(3):e305-15. https://doi.org/10.4317/medoral.20347.
9. John A, Peter T. Strategies to improve diagnosis and risk assessment for oral cancer patients. Faculty Dent J. 2020;11(3):122-7. https://doi.org/10.1308/rcsfdj.2020.97.
10. Chhabra N, Chhabra S, Sapra N. Diagnostic modalities for squamous cell carcinoma: an extensive review of literature-considering toluidine blue as a useful adjunct. J Maxillofac Oral Surg 2014;14(2):188-200. https://doi.org/10.1007/S12663-014-0660-6.
11. Kusukawa J, Suefuji Y, Ryu F, Noguchi R, Iwamoto O, Kameyama T. Dissemination of cancer cells into circulation occurs by incisional biopsy of oral squamous cell carcinoma. J Oral Pathol Med. 2000;29(7):303-7. https://doi.org/10.1034/J.1600-0714.2000.290703.X.
12. Crowley E, di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nature Rev Clin Oncol. 2013;10(8):472-84. https://doi.org/10.1038/NRCLINONC.2013.110.
13. Sahibzada HA, Khurshid Z, Khan RS, Naseem M, Siddique KM, Mali M, et al. Salivary IL-8, IL-6 and TNF-α as potential diagnostic biomarkers for oral cancer. Diagnostics (Basel). 2017 Apr 9;7(2):21. https://doi.org/10.3390/diagnostics7020021.
14. Strimbu K, Tavel JA. What are Biomarkers? Curr Opin HIV AIDS. 2010 Nov;5(6):463-6. doi: 10.1097/COH.0b013e32833ed177.
15. Aro K, Kaczor-Urbanowicz K, Carreras-Presas CM. Salivaomics in oral cancer. Curr Opin Otolaryngol Head Neck Surg. 2019;27(2):91-7. https://doi.org/10.1097/MOO.0000000000000502.
16. Carpenter GH. The secretion, components, and properties of saliva. Annu Rev Food Sci Technol. 2013:4:267-76. doi:10.1146/annurev-food-030212-182700.
17. Cheng J, Nonaka T, Wong DTW. Salivary exosomes as nanocarriers for cancer biomarker delivery. Materials (Basel). 2019;12(4):654. https://doi.org/10.3390/MA12040654.
18. Shah S. Salivaomics: The current scenario. J Oral Maxillofac Pathol. 2018 Sep-Dec;22(3):375-381. doi:10.4103/jomfp.JOMFP_171_18.
19. Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. Int J Oral Sci. 2016;8(3):133-7. https://doi.org/10.1038/ijos.2016.38.
20. Ai JY, Smith B, Wong DTW. Bioinformatics advances in saliva diagnostics. Int J Oral Sci. 2012;4(2):85-7. https://doi.org/10.1038/ijos.2012.26.
21. Poornima G, Kumar TSM. Genomic alphabets of saliva as a biomarker in oral cancer. J Indian Acad Oral Med Radiol. 2017;29(4):300. https://doi.org/10.4103/JIAOMR.JIAOMR_90_16.
22. Momen-Heravi F, Trachtenberg AJ, Kuo WP, Cheng YS. Genomewide study of salivary MicroRNAs for detection of oral cancer. J Dent Res. 2014 Jul;93(7 Suppl):86S-93S. doi:10.1177/0022034514531018.
23. Harrandah AM, Fitzpatrick SG, Smith MH, Wang D, Cohen DM, Chan EKL. MicroRNA-375 as a biomarker for malignant transformation in oral lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(6):743-752.e1. https://doi.org/10.1016/J.OOOO.2016.07.022.
24. Gai C, Camussi F, Broccoletti R, Gambino A, Cabras M, Molinaro L, et al. Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma. BMC Cancer. 2018 Apr 18;18(1):439. doi:10.1186/s12885-018-4364-z.
25. Zahran F, Ghalwash D, Shaker O, Al-Johani K, Scully C. Salivary microRNAs in oral cancer. Oral Dis. 2015 Sep;21(6):739-47. doi:10.1111/odi.12340.
26. Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. Oral Dis. 2010;16(4):360-4. https://doi.org/10.1111/J.1601-0825.2009.01646.X.
27. Salazar-Ruales C, Arguello J-V, López-Cortés A, Cabrera-Andrade A, Garc-A-Cárdenas JM, Guevara-Ram-Rez P, et al. Salivary MicroRNAs for early detection of head and neck squamous cell carcinoma: a case-control study in the high altitude Mestizo Ecuadorian population. Biomed Res Int. 2018 Nov 21:2018:9792730. https://doi.org/10.1155/2018/9792730.
28. Li Y, St. John MAR, Zhou X, Kim Y, Sinha U, Jordan RCK, et al. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res. 2004;10(24):8442-50. https://doi.org/10.1158/1078-0432.CCR-04-1167.

29. 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 Apr;21(4):664-72. doi:10.1158/1055-9965.EPI-11-1093.

30. Gleber-Netto FO, Yakob M, Li F, Feng Z, Dai J, Kao HK, et al. Salivary biomarkers for detection of oral squamous cell carcinoma in a Taiwanese population. Clin Cancer Res. 2016 Jul 1;22(13):3340-7. doi:10.1158/1078-0432.CCR-15-1761.

31. Arellano-Garcia ME, Hu S, Wang J, Henson B, Zhou H, Chia D, et al. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. Oral Dis. 2008;14(8):705-12. https://doi.org/10.1111/J.1601-0825.2008.01488.X.

32. St. John MAR, 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 Aug;130(8):929-35. doi:10.1001/archotol.130.8.929.

33. 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):51-5. https://doi.org/10.1016/j.oraloncology.2010.10.009.

34. Juretić M, Cerović R, Belušić-Gobić M, Pršo IB, Kqiku L, Špalj S, et al. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions. Folia Biol (Praha). 2013;59(2):99-102. https://europepmc.org/article/med/23746176.

35. Sahebjamee M, Eslami M, Atarbashimoghadam F, Sarafnejad A. Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008 May 1;13(5):E292-5.

36. Martin JL, Neil Gottehrer N, Zalesin H, Hoff PT, Shaw M, Clarkson JHW, et al. Evaluation of salivary transcriptome markers for the early detection of oral squamous cell cancer in a prospective blinded trial. Compend Contin Educ Dent. 2015 May;36(5):365-73.

37. Rapado-González Ó, López-López R, López-Cedrún JL, Triana-Martínez G, Muinelo-Romay L, Suárez-Cunqueiro MM. Cell-Free microRNAs as Potential Oral Cancer Biomarkers: From Diagnosis to Therapy. Cells. 2019 Dec 17;8(12):1653. doi:10.3390/cells8121653.