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Early Screening of Oral Premalignant and Malignant Lesions Using Salivary Biomarkers

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KEYWORDS
Biomarkers; Early detection; Oral cancer; Non-invasive screening

ABSTRACT

Background and Aim: Early identification of oral cancer may enhance treatment results dramatically. Non-invasive screening techniques that use salivary biomarkers have potential, although more validation is necessary. The primary objective of this research was to ascertain and assess microRNAs and interleukins as potential biomarkers that might be used for the prompt identification of oral pre malignant lesions and oral cancer.

Material and methods: A case-control study was undertaken, including saliva samples collected from 12 patients diagnosed with oral squamous cell carcinoma (OSCC), 10 patients diagnosd with leukoplakia, as well as a reference group of 20 healthy individuals. Demographic and clinical information was gathered. The measurement of microRNA-31 expression was performed using qRT-PCR. The IL-6 and IL-8 concentrations were measured using ELISA. The levels of the biomarkers under investigation were assessed in all groups, and all the data was provided as mean values and standard deviation.

Results: The findings of this study indicate that biomarkers interleukin-8 (IL-8), IL-6, and miR-31exhibited a statistically significant increase in oral cancer patients when compared to Leukoplakia and control group.

Conclusion: In conclusion, it is evident that salivary biomarkers IL-8, IL-6, and miR-31 exhibit promising characteristics as innovative early detection indicators for oral cancer and for monitoring oral premalignant lesions. Further validation is necessary for non-invasive screening that utilizes these biomarkers; however, it shows potential for enhancing rates of early detection.

INTRODUCTION

Oral cancer is a recognized disease identified by proliferation of malignant cells in the oral cavity, often affecting areas such as lips, floor of the mouth, gingiva, cheek, tongue, or palate. Oral cancer constitutes about one-third of the total cancer incidences globally, with India contributing to around 30% of these instances (Borse et al., 2020). The primary manifestations of oral cancer often include a persistent ulceration inside the oral cavity and persistent pain that is resistant to alleviation. Additional symptoms include the presence of a protuberance or increased density in the buccal region, the occurrence of white or red patches on the gingiva, tongue, and other intraoral areas, alongside a persistent pharyngeal discomfort and challenges in mastication and deglutition (Umapathy et al., 2022).

Reducing risk factors for oral cancer may help, such as drinking alcohol and smoking chewable or smokeless cigarettes. Furthermore, raising awareness of these issues can aid in the prevention of oral cancer. Multiple variables contribute to the onset of OSCC and result in both genetic and epigenetic changes. Precursor lesions of oral cancer may appear as red or white lesions, difficulty in opening the mouth, and a burning feeling inside the oral cavity. It is important to remember that a histological analysis of these lesions may show dysplastic characteristics. These conditions are categorized as oral potentially malignant disorders (OPMDs). The prognosis of OPMD and the patient's chances of survival depend

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critically on prompt identification of the condition in its early stages. Failure to identify OPMD promptly may have life-threatening consequences and lead to heightened morbidity rates (Chaturvedi et al., 2019). Furthermore, it is significant to highlight that oral cancer not only causes a considerable number of deaths but also has a significant impact on productivity due to premature mortality in developing nations (Pearce et al., 2018).

The most often used methods for identifying oral cancer and OPMDs, such as leukoplakia and erythroplakia, are visual examination and biopsy. At present, the diagnosis of oral cancer involves the use of intrusive procedures, such as the extraction of tissue from the affected region for biopsy purposes (Montero and Patel, 2015; Pollaers et al., 2018). Various approaches have been used, either alone or in conjunction with supplementary investigative techniques, to differentiate between OSCC and normal oral mucosa. The aforementioned approaches exhibit intrusive characteristics, substantial time demands, elevated prices, extensive dependency on human labor, and reliance on the investigator's degree of competence. The lesion may remain undetected unless a specialist diagnostic test is conducted. Biomarkers in shed cells, tissue specimens, or other body fluids such as saliva, blood, semen, and urine are present in a restricted concentration. In a person with excellent health, the presence of these markers is minimal. However, as the sickness advances, their quantity increases (Umapathy et al., 2022).

Oral fluid-based biomarkers are employed within the field of dentistry for the purpose of diagnosing conditions such as periodontitis, oral cancer, and caries. This depends on detecting samples of saliva and gingival crevicular fluid (GCF) (Lakshmi et al., 2017). Numerous biomarker types, including DNA, RNA, and protein biomarkers, are essential for the prompt detection of oral cancer (Awasthi, 2017). Studying the toxicity of salivary glands on oral fluid-based biomarkers is crucial since it can be brought on by exposure to dangerous substances, radiation therapy, or specific pharmaceuticals, among other things (Shamel et al., 2021; Bakr et al., 2022). Salivary glands may display changed functioning and secretion patterns when they are poisoned (Mansy et al., 2020; Ashraf et al., 2020). The composition of saliva and GCF may alter as a result, which may have an impact on the accuracy of biomarker detection in dentistry. Furthermore, prolonged inflammation and tissue damage brought on by the toxicity-induced damage to the salivary glands may encourage the generation of particular biomarkers linked to oral illnesses, such as oral cancer (Al Ankily et al., 2020).

Cytokines, including proinflammatory, anti-inflammatory, and chemokines, have the potential to be released by both the tumor and the surrounding lymphocytes. These cytokines might serve as biomarkers for the prompt identification of oral cancer (Chiamulera et al., 2021). In studies conducted by Arduino et al. (2015) and Rao et al. (2016), the involvement of inflammatory cytokines in controlling the pathophysiology of tumor growth and progression in OSCC has been demonstrated in detail. According to Arellano-Garcia et al. (2008), they might serve as indicators of oral cancer. Pro-inflammatory cytokines include both Interleukin (IL)-8 and IL-6. According to Lisa Cheng et al. (2014), they may be present in serum and saliva as well as other tissues. Both are thought to be biomarkers for a number of illnesses, including malignancies such as hepatocellular carcinoma (Shakiba et al., 2018) and oral lichen planus (Mozaffari et al., 2018). The diverse cytokine IL-6 has a major impact on the growth and differentiation factors of a number of cell types, including endothelial cells, neurons, T- and B-cells, and osteoclasts. According to Rajkumar et al. (2014), IL-8 is a chemotactic cytokine that is necessary for drawing in immune cells.

It has been established that IL-6 and IL-8 may be valuable biomarkers and are important factors in the pathogenicity of OSCC. Previous studies have demonstrated a connection between increased IL-6 levels and the onset of wasting, hypercalcemia, and cachexia, as well as the stimulation of immune unresponsiveness. These clinical manifestations are common in patients with OSCC who do not have a good prognosis (Oka et al. 1994). In order to promote angiogenesis—the creation of new blood vessels—proliferation—the division of cells—and chemotaxis—the movement of granulocytes and macrophages toward specific targets—the cytokine IL-8 is necessary. These biological processes play a particularly significant role in the stromal environment of OSCC. Rezaei and colleagues (2019) have reported that patients with OSCC have higher-than-normal blood and salivary IL-6 and IL-8 concentrations.

One of the best studied genetic components present in different body fluids is microRNAs, or miRNAs. These

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are small RNA molecules that aren't involved in protein coding. They play a part in controlling coding genes and usually have 19–25 nucleotides (Zhao et al., 2022). Tachibana et al. (2016) conducted a study that revealed a significant difference in plasma miR-223 levels between patients with gingival squamous cell carcinoma (GSCC) and the control group. The researchers came to the conclusion that miR-223 might be used as a GSCC diagnostic biomarker. It might also function as a tumor suppressor by preventing cell growth and encouraging cell death. According to Wen-li et al. (2015), plasma miR-125b demonstrated potential as an OSCC biomarker. The expression of plasma miR-125b was significantly higher in individuals with OSCC than in a reference group of healthy individuals. Additional research has demonstrated that the combined assessment of plasma miRNAs could be a useful technique for OSCC diagnosis. According to research by Lu et al. (2015), the simultaneous assessment of miR-196a and miR-196b levels demonstrated exceptional sensitivity, specificity, and accuracy in forecasting the course of oral cancer.

Therefore, the present paper presents a thorough examination of the advancements and developments in numerous biomarkers that are employed in the early detection of oral cancer and OPMDs.

MATERIAL AND METHODS

Study Design

Case-control study was conducted to evaluate the potential salivary biomarkers for the early identification and diagnosis of Leukoplakia and OSCC. The study included obtaining saliva samples from three distinct groups: 12 people diagnosed with OSCC, 10 individuals diagnosed with leukoplakia, and a control group of 20 healthy individuals. Prior to the initiation of the investigation, ethical approval was acquired from the appropriate institutional review board.

Patients Selection

A total of 12 individuals with OSCC and 10 patients with leukoplakia were selected from various outpatient clinics. The control group included twenty persons who were in good health and had no previous cases of oral cancer. These people were selected to match the experimental group in terms of gender, age, and other pertinent demographic variables. The inclusion criteria for both patient groups consisted of an established diagnosis of Leukoplakia and OSCC, determined via histological investigation. The exclusion criteria for all groups included persons with a prior history of other malignancies or systemic illnesses that might potentially impact biomarker concentrations. Data pertaining to demographics and clinical characteristics were gathered from the three cohorts, involving gender, age, smoking history, alcohol intake, and familial cancer predisposition. Each of the participants willingly signed the permission form authorized by the institutional review board, consenting to provide saliva samples for the investigation.

Collection of Saliva

Following Gai et al. (2018), unstimulated saliva was gathered from both the experimental groups and the healthy group in the morning, since it is the optimal period for non-invasive saliva gathering. Specimens were obtained by the method of passive salivation, and individuals were instructed to clean their mouth cavities with water before collection. The specimens were maintained in a manner that ensured their integrity and prevented any deterioration until they could be further analyzed.

RNA Isolation from saliva

The samples were thawed using an ice bath and thereafter subjected to centrifugation at a speed of 1500 revolutions per minute (rpm) at a temperature of 4 °C for a duration of 10 minutes. Total RNA was isolated from the sample based on a modified protocol from the manufacturer (mirVANA PARIS kit).

Examination of the expression of miRNA by qRT-PCR

The expression of miRNA was examined using qRT-PCR. The TaqMan® MicroRNA Reverse Transcription Kit from Applied Biosystem was used to convert total RNA into cDNA. The cDNA was then pre-amplified utilizing Megaplex™ RT Primers from Thermo Fisher Scientific, utilizing a Biometra Thermal Cycler. The evaluation of miR-31 expression across all analyzed groups was conducted. In order to guarantee accurate quantitation findings, every specimen was analyzed twice. The 2-ΔΔct method was used to normalize the results of miRNA evaluation, employing RNU6B as a

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baseline control. A quantitative reverse transcription polymerase chain reaction (qRT-PCR) study was carried out utilizing an ultimate reaction mixture volume of 20 µl. The mixture included 10 nanograms of cDNA, a miRNA-specific primer at a concentration of 20 nanomolar, nuclease-free water from Qiagen, 10 microliters of QuantiTect SYBR Green PCR Master Mix from Qiagen, and a miScript Universal Primer at a concentration of 50 nanomolar from Qiagen. The investigation was conducted with the Real-Time Thermal Cycler StepOne Plus, by Applied Biosystems situated in Foster City, USA.

Enzyme-Linked Immune-Sorbent Assay (ELISA)

The quantities of IL-8 and IL-6 were measured in isolated saliva using the PicoKine™ ELISA kit from MyBiosource.com. The procedure was executed in accordance with the instructions provided in the handbook.

Statistical analysis

The salivary tested biomarkers information was analyzed utilizing the Statistical Package for Social Sciences (SPSS) 10.3 software, and the results were given as mean values and standard deviation. The mean values among the study groups were compared using one-way analysis of variance test (ANOVA) and Student's t-test. A P-value was deemed highly statistically significant if it was less than or equal to 0.01, and significant if it was less than or equal to 0.05.

RESULTS

The analysis of demographic and clinical data revealed that the oral cancer group had elevated rates of smoking in comparison to the control group. An increased risk of oral cancer was seen in individuals of advanced age and those with a family history of cancer. The median age of control participants was 45 years with a variation of 10 years, whereas the median age of leukoplakia patients was 52 years with an 8-year variation and that of OSCC patients was 58 years with a 7-year variation. According to the data, the OSCC group had more current smokers than the leukoplakia group and the control group. This confirms the fact that smoking tobacco is a substantial risk factor for developing OSCC. Ulcerative OSCC was the most prevalent clinical type, followed by erythroplakic then exophytic (Table 1).

Table 1. The demographic information pertaining to reference participants, leukoplakia, and OSCC patients.

Demographic Data	Control Subjects	Leukoplakia Patients	OSCC Patients
Age (median ± SD)	45 ± 10	52 ± 8	58 ± 7
Gender			
Male	10	7	8
Female	10	3	4
Tobacco Smoking Status			
Non-smoker	15	4	3
Current Smoker	5	6	9
Clinical Form			
Ulcerative			8
Exophytic			1
Erythroplakic Leukoplakic		10	3
Verrucous			0
Mixed			0
Histological Tumor Differentiation			
Well Differentiated			6
Not Well Differentiated			6

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Examination of the expression of miRNA by qRT-PCR

Through qRT-PCR array analysis of miRNA expression, it was determined that OSCC patients had an upregulation of MiR-31 in comparison to the Leukoplakia and healthy groups. A substantial and statistically significant rise in miR-31 level was identified in patients with OSCC contrasted to both the leukoplakia group and the control group ($p \leq 0.001$), as seen in Figure 1 and Table 2.

Our investigation revealed that the expression of miR-31 was elevated in high grade OSCC (5.979±0.916) compared to low grade cases (4.6341±0.583). Nevertheless, there was no discernible statistical disparity seen across various classes of OSCC.

Table 2. The miR-31 concentrations' mean values in isolated salivary samples from each category.

Biomarker	OSCC Group	Leukoplakia Group	Control Group
miR-31	4.7 ± 0.5	2.6 ± 0.4	1.1 ± 0.3

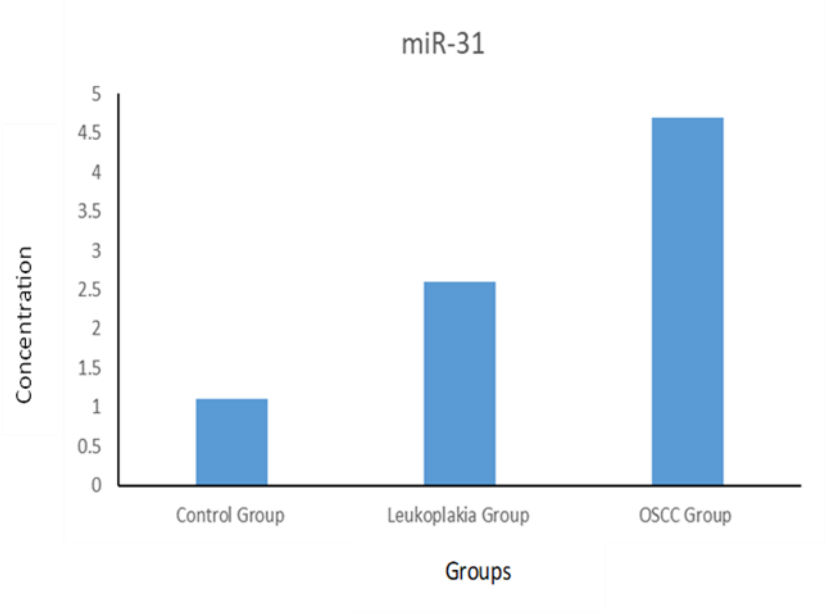

Figure 1. The mean values of miR-31 concentrations.

The expression of IL-8 and IL-6 by ELISA

The levels of salivary IL-6 showed a substantial and statistically significant rise in patients with OSCC contrasted to those with leukoplakia and the control groups ($p \leq 0.001$). Furthermore, the study revealed a noteworthy elevation in salivary IL-8 levels among patients with OSCC in comparison to patients with leukoplakia and the control group ($p \leq 0.001$), as seen in Table 3 and Figure 2.

Table 3. The IL-8 and IL-6 concentrations' mean values in salivary samples from each category.

Biomarker	OSCC Group	Leukoplakia Group	Control Group
IL-6	245.5 ± 40.3	122.64 ± 21.38	33.2 ± 1.8
IL-8	1194.7±285.0	815.40±123.04	220.3 ± 62.2

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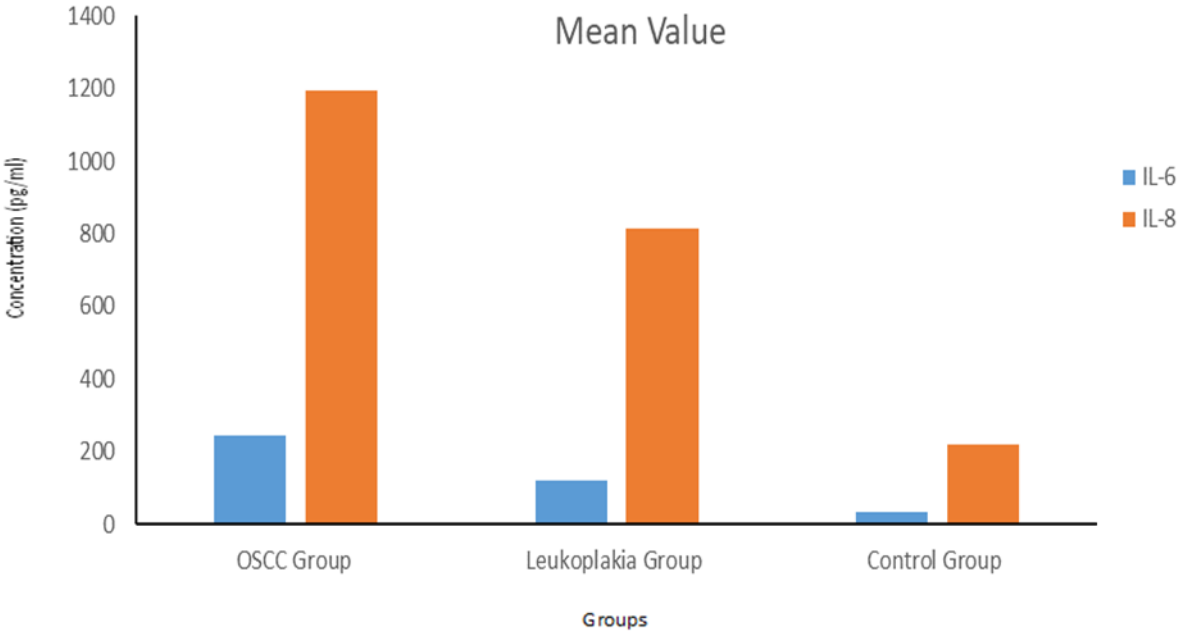

Figure 2. The mean values of IL-8 and IL-6 concentrations.

The findings of this study provide substantiation that biomarkers IL-6, IL-8, and miR-31 have the potential to function as innovative indicators for the early identification of oral cancer, particularly when used together.

DISCUSSION

Management and subsequent monitoring of patients with OSCC provide an enormous obstacle for oral surgery specialists and oncologists. The enhancement of diagnostic and prognostic capabilities for individuals with oral cancer necessitates the imperative growth of novel and precise biomarkers. Saliva maintains continuous interaction with oral tissues and may be readily acquired using noninvasive means. These characteristics provide it a favorable resource of samples for the exploration of possible biomarkers (Khurshid et al., 2018).

Despite the presence of many enzymes, including ribonucleases responsible for RNA destruction, saliva has been shown to retain detectable levels of distinct RNAs. This observation suggests the existence of unidentified mechanisms that safeguard salivary RNAs against degradation (Park et al., 2006; Nagler, 2009). Moreover, it is worth noting that RNAs produced in cells of various body tissues might potentially migrate via the systemic circulation and then be excreted into saliva (Park et al., 2006). The investigation into the composition of saliva appears to have potential for the surveillance and diagnosis of not only oral ailments and well-being, but also systemic disorders and overall health (Hu et al., 2008). MiRNAs may maintain resilience in many physiologic settings, such as saliva, plasma, and other body fluids, due to their inherent structural stability (Chen et al., 2008). The aforementioned attribute renders miRNA a superior biomarker compared to other macromolecules present in body fluids, when it comes to identifying and diagnosing certain malignant disorders (Liu et al., 2010; Zubakov et al., 2010).

The findings of our investigation demonstrated a significant elevation in miR-31 levels in saliva samples obtained from patients diagnosed with OSCC in comparison to those obtained from leukoplakia patients and persons without any known health conditions. Although there are other factors that may impact the presence of salivary miR-31, the observation of much greater levels of salivary miR-31 in OSCC group compared to Leukoplakia and control groups suggests a substantial regional role for miR-31 to the composition of saliva in oral cancer.

These results are consistent with the study done by Lin et al. (2022), which showed that miR-31 has a significant

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role in the progression and development of OSCC. The study also revealed a significant upregulation of miR-31 expression in OSCC patients' tumor tissue, plasma, and saliva. The significance of miR-31 in the development of OSCC can be attributed to its ability to create a complex network involving target genes and signaling cascades, such as the EGF-AKT signaling axis, the ERK-MMP9 cascade, and Wnt signaling. Our study's findings suggest that miR-31 might be a helpful biomarker for OSCC diagnosis, prognosis prediction, and assessment of treatment response. Further investigation is required to obtain a more profound comprehension of the molecular mechanisms that underlie the advancement of OSCC induced by miR-31. Moreover, it is critical to look into the possibility of miR-31 as a prognostic tool for predicting the probability that OPMDs will advance to OSCC.

The current study found that the saliva of those with OSCC had a statistically significant and elevated level of IL-6 and IL-8 when compared to people with leukoplakia and a healthy reference group. Our findings are consistent with the research conducted by Goldoni et al. (2021), supporting the notion that IL-8 and IL-6 are very useful salivary markers for the early identification of oral cancer. Also, various proangiogenic and pro-inflammatory cytokines were found in saliva samples from patients with oral cancer and oral precancerous diseases at higher than normal levels. These cytokines included IL-8, IL-6, IL-1 and TNF-α. This was discovered by Poornima and Kumar (2017) who suggested that these cytokines may be employed as alternative markers for the progression of oral precancerous lesions to oral cancer. Moreover, they proposed that variations in salivary TNF-α and IL-6 levels could be a factor in the development of oral leukoplakia. According to Rai et al. (2010), the outcomes were attributed to a marked increase in cell proliferation and the initiation of biological mechanisms brought on by persistent inflammation, which resulted in the manifestation of irreversible DNA damage.

According to the findings of this study and the previously discussed functions of salivary cytokines, the significant rise in the generation of salivary IL-8 and IL-6 is regarded as a prominent characteristic of the development of cancer. These cytokines can serve as indicators for early identification of cancer and for evaluating the progression of cancerous transformation in OPMDs. Furthermore, the high correlation between the expression of the two different set of biomarkers suggests that employing both salivary markers together may give a more stronger prediction capacity.

CONCLUSION

The present work has successfully identified biomarkers IL-6, IL-8, and miR-31as very promising candidates for the early identification of oral cancer. The findings of this study provide support for the notion that these biomarkers have the potential to enhance the feasibility of non-invasive oral cancer screening as well as monitoring the activity of OPMDs. Although more validation studies are required, the findings are promising in terms of facilitating the creation of screening instruments to assist in the early identification of oral cancer leading to improved therapeutic results. Additional investigation is necessary to validate these results and facilitate the translation of biomarkers into tangible diagnostic applications.

REFERENCES

1. Al Ankily, M., Shamel, M., & Bakr, M. Epidermal Growth Factor Improves the Ultrastructure of Submandibular Salivary Glands of Streptozotocin Induced Diabetic Rats - A Qualitative Study. *International Journal of Medical and Dental Sciences*, 9(1), 1803.
2. Ashraf, B., Ghazy, D., & Shamel, M. (2020). Effects of aflatoxin B1 on the submandibular salivary gland of albino rats and possible therapeutic potential of *Rosmarinus officinalis*: a light and electron microscopic study. *F1000Research*, *9*,752.
3. Arduino, P. G., Menegatti, E., Cappello, N., Martina, E., Gardino, N., Tanteri, C., ... & Broccoletti, R. (2015). Possible role for interleukins as biomarkers for mortality and recurrence in oral cancer.
4. Arellano-Garcia, M. E., Hu, S., Wang, J., Henson, B., Zhou, H., Chia, D., & Wong, D. T. (2008). Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. *Oral diseases*, *14*(8), 705-712.
5. Awasthi, N. (2017). Role of salivary biomarkers in early detection of oral squamous cell

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www.jchr.org

JCHR (2023) 13(6), 2323-2331 | ISSN:2251-6727

carcinoma. *Indian Journal of Pathology and Microbiology*, *60*(4), 464.

6. Bakr, M.M., Al-Ankily, M.M., Shogaa, S.M., & Shamel, M. (2021). Attenuating Effect of Vitamin E against Silver Nano Particles Toxicity in Submandibular Salivary Glands. *Bioengineering*, *8*, 219.
7. Borse, V., Konwar, A. N., & Buragohain, P. (2020). Oral cancer diagnosis and perspectives in India. *Sensors International*, *1*, 100046.
8. Chaturvedi, P., Singh, A., Chien, C. Y., & Warnakulasuriya, S. (2019). Tobacco related oral cancer. *Bmj*, *365*.
9. Chen, X., Ba, Y., Ma, L., Cai, X., Yin, Y., Wang, K., ... & Zhang, C. Y. (2008). Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell research*, *18*(10), 997-1006.
10. Chiamulera, M. M. A., Zancan, C. B., Remor, A. P., Cordeiro, M. F., Gleber-Netto, F. O., & Baptistella, A. R. (2021). Salivary cytokines as biomarkers of oral cancer: A systematic review and meta-analysis. *BMC cancer*, *21*(1), 1-16.
11. Gai, C., Camussi, F., Broccoletti, R., Gambino, A., Cabras, M., Molinaro, L., ... & Arduino, P. G. (2018). Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma. *BMC cancer*, *18*(1), 1-11.
12. Goldoni, R., Scolaro, A., Boccalari, E., Dolci, C., Scarano, A., Inchingolo, F., ... & Tartaglia, G. (2021). Malignancies and biosensors: A focus on oral cancer detection through salivary biomarkers. *Biosensors*, *11*(10), 396.
13. Hu, S., Arellano, M., Boontheung, P., Wang, J., Zhou, H., Jiang, J., ... & Wong, D. T. (2008). Salivary proteomics for oral cancer biomarker discovery. *Clinical cancer research*, *14*(19), 6246-6252.
14. Khurshid, Z., Zafar, M. S., Khan, R. S., Najeeb, S., Slowey, P. D., & Rehman, I. U. (2018). Role of salivary biomarkers in oral cancer detection. *Advances in clinical chemistry*, *86*, 23-70.
15. Lakshmi, K. R., Nelakurthi, H., Kumar, A. S., & Rudraraju, A. (2017). Oral fluid-based biosensors: A novel method for rapid and noninvasive diagnosis. *Indian Journal of Dental Sciences*, *9*(1), 60-66.
16. Lin, X., Wu, W., Ying, Y., Luo, J., Xu, X., Zheng, L., Wu, W., Yang, S., & Zhao, S. (2022). MicroRNA-31: a pivotal oncogenic factor in oral squamous cell carcinoma. *Cell death discovery*, *8*(1), 140. https://doi.org/10.1038/s41420-022-00948-z
17. Lisa Cheng, Y. S., Jordan, L., Gorugantula, L. M., Schneiderman, E., Chen, H. S., & Rees, T. (2014). Salivary interleukin-6 and-8 in patients with oral cancer and patients with chronic oral inflammatory diseases. *Journal of periodontology*, *85*(7), 956-965.
18. Liu, C. J., Kao, S. Y., Tu, H. F., Tsai, M. M., Chang, K. W., & Lin, S. C. (2010). Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. *Oral diseases*, *16*(4), 360-364.
19. Lu, Y. C., Chang, J. T. C., Huang, Y. C., Huang, C. C., Chen, W. H., Lee, L. Y., ... & Cheng, A. J. (2015). Combined determination of circulating miR-196a and miR-196b levels produces high sensitivity and specificity for early detection of oral cancer. *Clinical biochemistry*, *48*(3), 115-121.
20. Mansy, M., Soliman, M., Mubarak, R., & Shamel, M. (2020). The role of exogenous epidermal growth factor on Ki-67 proliferation marker expression in the submandibular salivary gland of albino rats receiving doxorubicin. *F1000Research*, *9*,1393.
21. Montero, P. H., & Patel, S. G. (2015). Cancer of the oral cavity. *Surgical Oncology Clinics*, *24*(3), 491-508.
22. Mozaffari, H. R., Sharifi, R., Mirbahari, S., Montazerian, S., Sadeghi, M., & Rostami, S. (2018). A systematic review and meta-analysis study of salivary and serum interleukin-8 levels in oral lichen planus. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, *35*(6), 599-604.
23. Nagler, R. M. (2009). Saliva as a tool for oral cancer diagnosis and prognosis. *Oral oncology*, *45*(12), 1006-1010.
24. Oka, M., Yamamoto, K., Takahashi, M., Hakozaki, M., Abe, T., Iizuka, N., ... & Suzuki, T. (1996). Relationship between serum levels of interleukin 6, various disease parameters, and malnutrition in

Journal of Chemical Health Risks

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JCHR (2023) 13(6), 2323-2331 | ISSN:2251-6727

patients with esophageal squamous cell carcinoma. *Cancer research*, *56*(12), 2776-2780.

25. Park, N. J., Yu, T., Nabili, V., Brinkman, B. M., Henry, S., Wang, J., & Wong, D. T. (2006). RNAprotect saliva: An optimal room-temperature stabilization reagent for the salivary transcriptome. *Clinical chemistry*, *52*(12), 2303-2304.
26. Pearce, A., Sharp, L., Hanly, P., Barchuk, A., Bray, F., de Camargo Cancela, M., ... & Soerjomataram, I. (2018). Productivity losses due to premature mortality from cancer in Brazil, Russia, India, China, and South Africa (BRICS): A population-based comparison. *Cancer epidemiology*, *53*, 27-34.
27. Pollaers, K., Hinton-Bayre, A., Friedland, P. L., & Farah, C. S. (2018). AJCC 8th Edition oral cavity squamous cell carcinoma staging–Is it an improvement on the AJCC 7th Edition. *Oral oncology*, *82*, 23-28.
28. Poornima, G., & Kumar, T. S. M. (2017). Genomic alphabets of saliva as a biomarker in oral cancer. *Journal of Indian Academy of Oral Medicine and Radiology*, *29*(4), 300-305.
29. Rai, B., Kaur, J., Jacobs, R., & Singh, J. (2010). Possible action mechanism for curcumin in pre-cancerous lesions based on serum and salivary markers of oxidative stress. *Journal of oral science*, *52*(2), 251-256.
30. Rajkumar, K., Nandhini, G., Ramya, R., Rajashree, P., Kumar, A. R., & Anandan, S. N. (2014). Validation of the diagnostic utility of salivary interleukin 8 in the differentiation of potentially malignant oral lesions and oral squamous cell carcinoma in a region with high endemicity. *Oral surgery, oral medicine, oral pathology and oral radiology*, *118*(3), 309-319Rao, M., Ramesh, A., Adapa, S., Thomas, B., & Shetty, J. (2016). Correlation of salivary levels of interleukin-6 and albumin with oral squamous cell carcinoma. *Journal of Health Research and Reviews*, *3*(1), 11.
31. Rezaei, F., Mozaffari, H. R., Tavasoli, J., Zavattaro, E., Imani, M. M., & Sadeghi, M. (2019). Evaluation of serum and salivary interleukin-6 and interleukin-8 levels in oral squamous cell carcinoma patients: Systematic review and meta-analysis. *Journal of Interferon & Cytokine Research*, *39*(12), 727-739.
32. Shakiba, E., Ramezani, M., & Sadeghi, M. (2018). Evaluation of serum interleukin-6 levels in hepatocellular carcinoma patients: A systematic review and meta-analysis. *Clinical and Experimental Hepatology*, *4*(3), 182-190.
33. Shamel, M., Riad., D., & Al Ankily, M. (2021). Histological and ultrastructural study of silver nanoparticles toxicity and the possible protective effect of vitamin c on submandibular salivary glands of albino rats. *Int J Dentistry Oral Sci*, 8(4), 2166.
34. Tachibana, H., Sho, R., Takeda, Y., Zhang, X., Yoshida, Y., Narimatsu, H., ... & Iino, M. (2016). Circulating miR-223 in oral cancer: its potential as a novel diagnostic biomarker and therapeutic target. *PloS one*, *11*(7), e0159693.
35. Umapathy, V. R., Natarajan, P. M., Swamikannu, B., Moses, J., Jones, S., Chandran, M. P., & Anbumozhi, M. K. (2022). Emerging Biosensors for Oral Cancer Detection and Diagnosis—A Review Unravelling Their Role in Past and Present Advancements in the Field of Early Diagnosis. *Biosensors*, *12*(7), 498.
36. Wen-li, G. U., Dong-xia, Y. E., & Jing-jing, W. U. (2015). Expression and clinical significance of plasma microRNA-125b level in patients with oral squamous cell carcinoma. *Shanghai Journal of Stomatology*, *24*(1), 71.
37. Zhao, Y., Dhani, S., & Zhivotovsky, B. (2022). Unveiling caspase-2 regulation by non-coding RNAs. *Cell Death & Disease*, *13*(9), 834.
38. Zubakov, D., Boersma, A. W., Choi, Y., van Kuijk, P. F., Wiemer, E. A., & Kayser, M. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. *International journal of legal medicine*, *124*, 217-226.