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**ORIGINAL PAPER**

# Salivary tumour necrosis factor-alpha and receptor for advanced glycation end products as prognostic and predictive markers for recurrence in oral squamous cell carcinoma – a pilot study

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**ABSTRACT**

**Introduction and aim.** Tumour necrosis factor-alpha (TNF-α) belongs to the cytokine family TNF/TNFR. As a multifunctional cytokine, TNF-α plays a significant role in diverse and a variety of cellular events such as cell survival, proliferation, differentiation, and death. As a pro-inflammatory cytokine, TNF-α acts as a bridge between inflammation and carcinogenesis. Receptor for advanced glycation end products (RAGE) are cellular receptors belonging to the immunoglobulin superfamily. As one of the primary mediators of innate immunity, acute and chronic inflammatory disorders, and certain cancers, RAGE signaling plays an important role. The aim of the present study is to analyze the prognostic significance of salivary TNF-α and RAGE in oral squamous cell carcinoma.

**Material and methods.** A study was conducted testing saliva samples collected from ten patients with well-differentiated and moderately differentiated oral squamous cell carcinomas. To determine the levels of TNF-α and RAGE in unstimulated saliva from patients, an ELISA kit from RAY BIOTECH was used for the study, and the readings were read at 450 nm. Statistical analysis was performed using SPSS software. Version 23 of SPSS was used to plot the standard curve. Statistical comparisons were done using Mann-Whitney U test and ROC analysis.

**Results.** Salivary TNF-α and RAGE in patients were considered to be induced by radiotherapy at a higher level in moderately differentiated squamous cell carcinoma when compared to well differentiated squamous cell carcinoma. Thus, there is an increase in the induced Salivary TNF-α and RAGE levels by radiotherapy with increase in the histological stages of oral squamous cell carcinoma. The statistical analysis also proved the same.

**Conclusion**. Hence salivary TNF-α and RAGE may be used as a biomarker for oral cancer to predict the prognosis.

**Keywords**. biomarker, oral squamous cell carcinoma, prognosis, RAGE, saliva, TNF-α

## Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor that is located in the upper part of the digestive system. 54010 new cases of oropharyngeal cancer were recorded in 2021 based on cancer statistics. Most of these cancers arise in the mouth, on the tongue, in the tonsils and oropharynx, in the gums, on the floor of the mouth, and elsewhere in the oral cavity.[1] They can be found in the lips and minor salivary glands of the oral cavity. Most people diagnosed with these cancers are older than 63,

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though some younger people can also be affected. About 20% of cases occur in people younger than 40. Whites are slightly more likely to be affected than Blacks. According to the National Cancer Institute, the lifetime risk of developing oral cavity and oropharyngeal cancer is about 1 in 60 (1.7%) for men and 1 in 140 (0.71%) for women. The risk of mouth and throat cancer is also influenced by a number of other factor.[2] During the past 20 years, the overall rate of new cases of oral cavity and oral squamous cell carcinoma has only slightly increased. Yet during this same time, both women and men have seen an increase in OSCC associated with human papillomavirus (HPV) infection. Over the last 30 years, these cancers have experienced a decline in death rates.[3]

The assay for salivary biomarkers has developed tremendously in recent years. Many omics technologies, including proteomics and transcriptomics, have been applied to analyze salivary constituents.[4] Among the newer additions to the omics field, metabolomics examines biofluids, cells, and tissues for metabolites. Molecular substances such as these are substrates, intermediates, or products of biochemical reactions.[5] Cells, tissues, and organisms respond physiologically or pathologically to such molecules depending on their genetic properties and environmental exposure.[6]

Tumour necrosis factor – alpha (TNF-α) is a member of the TNF/TNFR cytokine family.[7] TNF-α is mainly involved in the maintenance of the source and homeostasis of the basic immune system, inflammation, and host defense.[8] It is a type II transmembrane protein with an intracellular N terminus. It has a particular signaling potential both as a membrane-integrated protein and as a soluble cytokine released after proteolytic cleavage.[9-11] TNF-$\alpha$ has regulatory soluble functions on various crucial physiological processes such as synaptic plasticity, learning and memory, and astrocyte-induced total synaptic strengthening TNF-α is a multifunctional cytokine that plays an important role in diverse and a variety of cellular events such as cell survival, proliferation, differentiation, and death.[12,13] As a particular pro-inflammatory cytokine family, Tumor necrosis factor is secreted by the inflammatory cells, which may be involved in inflammation and leads to carcinogenesis.

As a major proinflammatory cytokine, TNF-α is able to act as an endogenous tumor promoter to bridge inflammation and carcinogenesis. In regard to cancer, TNF-α plays a double role.[14] On one hand, TNF-α could be an endogenous tumor promoter, because TNF stimulates cancer cells' growth, proliferation, invasion and metastasis, and tumor angiogenesis. On the other hand, TNF-α could be a cancer killer.[15] The property of TNF-α in inducing cancer cell death makes it potential cancer therapeutic, although much effort is needed to decrease its toxicity for systematic TNF-α administration. It is also aimed at sensitizing cancer cells to TNF-α induced apoptosis by inhibiting survival signals such as NF-κB, by combined therapy.[16]

Receptor for advanced glycation end-products (RAGE) is a cell surface receptor that belongs to the immunoglobulin (Ig) superfamily. RAGE signaling plays a central role within the inflammatory response, mediating aspects of innate immunity, acute and chronic inflammatory conditions, and certain cancers.[17-20] Activated RAGE recruits extracellular signal-regulated kinase–1 and –2 (ERK-1/2), leading to downstream activation of NF-κB via the MAP kinase pathway.[21]

The human RAGE gene has a short 3' UTR, eleven exons, and a 5' flanking region that regulates its transcription. It is found on chromosome 6 in the class III area of the major histocompatibility complex. RAGE mediates physiological and pathological consequences by interacting with a number of ligands, each of which is linked to a specific illness.[22] RAGE plays multiple roles in cancer cells; RAGE-HMGB1 interaction stimulates tumor invasiveness and growth.[20] Moreover, HMGB1-DNA complexes trigger the association of RAGE with Toll-like receptor 9, which is important for the immune reaction to pathogens.[18] The whole saliva represents a posh balance among local and systemic sources. This enables the appliance of saliva within the diagnosis not just for exocrine gland disorders but also for oral diseases and systemic conditions. The role of saliva as a diagnostic tool in detecting oral squamous cell carcinoma is currently under research, the prognostic value of RAGEs in head and neck cancers looks promising.

The role of TNF- α and RAGEs in head and neck cancers and their prognostic use is now being studied off-lately, but the expression of RAGEs within the saliva in post-radiation therapy is not yet elucidated as immediate cellular damage after irradiation is supposed to result in cytokine-mediated multicellular interactions with induction and progression of inflammatory and fibrotic tissue reactions.

## Aim

Thus, this study aims is to analyze the prognostic significance of radiation induced salivary TNF- α and RAGE in oral squamous cell carcinoma with the following objectives: i) to elucidate the expression and levels of TNF-α and RAGE expression within the salivary samples of post-radiation oral squamous cell carcinoma of various stages, ii) to determine if levels increase with increasing OSCC histological stages after radiotherapy treatment, and iii) to know the efficacy of the TNF-α and RAGE as salivary biomarkers in OSCC.

## Material and methods

### *Ethical approval*

Prior to the initiation of the study, clearance was obtained by the Scientific Review Board with the Ethical approval number IHEC/SDC/BDS/1977/01.

***Study design***

An *in vivo* comparative study was conducted on saliva samples from randomly selected ten (n=10) patients who presented with oral squamous cell carcinomas, well-differentiated (5) and moderately differentiated (5), at the tertiary cancer care center for radiotherapy over a period of 3months from February to May 2021. A saliva sample was collected and tested after the patient underwent five rounds of regular radiotherapy treatment. The study was non-invasive and easy to perform without much inconvenience to patients. However, the sample size was limited, the test validation was done by an expert pathologist.

***Criteria for selection of study subjects***

Patients who were diagnosed to have well-differentiated and moderately differentiated oral squamous cell carcinoma, had completed all five cycles of radiotherapy treatment for the same were included in the study. As the objective of the study includes the histological staging alone, the clinical staging was not included in the present study . It was also ensured that patients with systemic comorbidities or terminally ill patients were not taken up for the study. The patients who developed radiation induced mucosal changes were excluded. As the expression of salivary TNF-α and RAGE (Ghrelin) in normal healthy individuals stated in the previous studies as at a non-detectable amount, we excluded the healthy controls from our study.[23]

All the subjects included in the study belonged to the same ethnic group. Informed consent was obtained from the study subjects for inclusion in the study and it was also ensured that the subject's anonymity was maintained. All the participants completed a questionnaire covering medical, residential, and occupational history.

***Sample collection***

10 saliva samples were collected from patients with post-radiotherapy oral cancer. Unstimulated saliva from the patients was collected in Eppendorfs for a volume of 1ml. Then it was stored at -20°C. During the procedure, it was thawed and centrifuged. Samples were collected during the timeframe of 4 months in the year 2021.

***Principle of the test***

ELISA is based on the competitive binding technique in which the TNF-α and RAGE (Ghrelin) present in the sample competes with a fixed amount of horseradish peroxide (HRP)-labeled TNF-α and RAGE (Ghrelin) on a human monoclonal antibody. Standards and samples are pipetted into the wells and TNF-α and RAGE (Ghrelin) present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated anti-human TNF-α and RAGE (Ghrelin) antibody was added.

After washing away unbound biotinylated antibody, HRP conjugated streptavidin is pipetted into the wells. The wells were washed again, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF-α and RAGE (Ghrelin) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

***Reagent preparation***

All reagents and samples were brought to room temperature (18-25°C) before use. Also, Assay Diluent B (Item E) should be diluted to 5-fold with deionized or distilled water before use. For dilution of samples, Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. The suggested dilution for normal serum/plasma is 2 - 20 fold. For the preparation of the standard, a vial of Item C was briefly spun. 400 µL of Assay Diluent A (for serum/ plasma samples) was added to Item C vial to prepare the 50ng/ml standard. The powder was dissolved thoroughly by a gentle mix.

15 µL TNF-α and RAGE (Ghrelin) standard (50 ng/ml) was added from the vial of Item C, into a tube with 485 µL Assay Diluent A or 1X Assay Diluent B to prepare a 1,500 pg/ml standard solution. 400 µL Assay Diluent A or 1X Assay Diluent B was pipetted into each tube. 1,500 pg/ml standard solution was used to produce a dilution series (shown below). Each tube was mixed thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B served as the zero standards (0 pg/ml). If the Wash Concentrate (20X) (Item B) contained visible crystals, it was warmed to room temperature and mixed gently until they dissolved.

20 ml of Wash Buffer Concentrate was diluted into deionized or distilled water to yield 400 ml of 1X Wash Buffer. Detection Antibody vial (Item F) was briefly spun before use. 100 µL of 1X Assay Diluent B (Item E) was added to the vial to prepare a detection antibody concentrate. This was then pipetted up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in relevant prior steps. The HRP-Streptavidin concentrate vial (Item G) was briefly spun and pipetted up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent B (Item E).

***Assay procedure***

All reagents and samples were brought to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Removable 8-well strips were labeled as appropriate for the experiment. 100 µL of each standard and sample was added to appropriate wells. These wells were then covered and incubated for 2.5 hours at room tempera-

ture with gentle shaking. The solution was discarded and washed 4 times with 1X wash solution. Each well was filled and washed with Wash Buffer (300 µl) using a Pipette. Complete removal of the liquid at each step is essential for good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted with clean paper towels. 100 µl of 1X prepared biotinylated antibody was added to each well. This was then incubated for 1 hour with gentle shaking. The solution was discarded and the wash was repeated. 100 µL of prepared Streptavidin solution was added to each well. This was then incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and the wash was repeated. 100 µL of TMB One-Step Substrate Reagent (Item H) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 µl of Stop Solution (Item I) was added to each well and read at 450 nm immediately.

***Calculation of results***

The mean absorbance was calculated for each set of duplicate standards, controls, and samples, and the average zero standard optical density was subtracted. The standard curve was plotted using SPSS software version 23, with standard concentration on the x-axis and absorbance on the y- axis. The best-fit straight line was drawn through the standard points.

***Sensitivity***

The minimum detectable dose of Human TNF-α and RAGE (Ghrelin) was determined to be 3 pg/ml. The minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluents buffer).

***Statistical analysis***

Statistical analysis was performed using SPSS software (IBM, Armonk, New York, United States). As the same size was less than 30, Mann-Whitney U test was performed to compare the results. Dependent variables include oral cancer while independent variables include age and sex.

## Result

***Demographic data***

The study included a total of n=10 participants who were divided into two groups. Group I consists of well differentiated squamous cell carcinoma (WDSCC) n=5 and Group II consists of moderately differentiated squamous cell carcinoma (MDSCC) n=5,

***Statistics***

Mann-Whitney U test was performed to compare the results and $p<0.05$ was considered significant and $p>0.05$ was considered not significant. Also ROC curve analysis was done with significance level <0.05.

***Salivary TNF-α in patients with WDSCC***

Among the 5 samples analyzed, all 5 showed the presence of TNF-α in salivary samples. The mean value of salivary TNF-α among Group I was 7.87±0.26 pg/dl with a median of 8 pg/dl.

***Salivary TNF-α in patients with MDSCC***

Among the 5 samples analyzed, all 5 showed the presence of TNF-α in salivary samples. The mean value of salivary TNF-α among Group II was 12.81±0.724 pg/dl with a median of 12.42 pg/dl.

***Salivary RAGE (Ghrelin) in patients with WDSCC***

Among the 5 samples of the saliva of patients with WDSCC analyzed, all 5 samples showed the presence of RAGE in the tissue samples. The mean value of RAGE among the WDSCC group a mean value of 0.684±0.296 pg.dl with a median of 0.83 pg/dl.

***Salivary RAGE (Ghrelin) in patients with MDSCC***

Among the 5 samples analyzed, all 5 samples showed the presence of RAGE in the tissue samples. The mean value of RAGE among the MDSCC group was 1.32±0.258 pg/dl with a median of 1.3 pg/dl.

***Comparison of TNF-α and RAGE (Ghrelin) among salivary samples of WDSCC and MDSCC***

The comparison between the groups shows statistical significance. A higher salivary TNF-α and RAGE (Ghrelin) was found among MDSCC patients when compared to those of WDSCC patients.

**Table 1**. Table showing the mean of the significance of the observations at the levels of $p<0.05$

| Salivary Markers | Group | Mean (pg/dl) | Std. Deviation (pg/dl) | Median (pg/dl) | Quartiles (Q1,Q3) (pg/dl) | p value (Non parametric analysis and ROC) |
|---|---|---|---|---|---|---|
| TNF-α (pg/dl) | WDSCC | 7.878 | 0.265 | 8 | 7.66,8.03 | 0.009 |
| | MDSCC | 12.81 | 0.72 | 12.42 | 12.31,13.50 | (<0.05) |
| RAGE (Ghrelin) (pg/dl) | WDSCC | 0.684 | 0.296 | .83 | 0.40,0.895 | 0.009 (<0.05) |
| | MDSCC | 1.32 | 0.258 | 1.3 | 1.1,1.55 | |

Both TNF-α and RAGE (Ghrelin) levels were high in patients with MDSCC.

The median value of TNF-α in well differentiated carcinoma (8 pg/dl) were significantly lower than the values of moderately differentiated carcinoma (12.42 pg/dl).

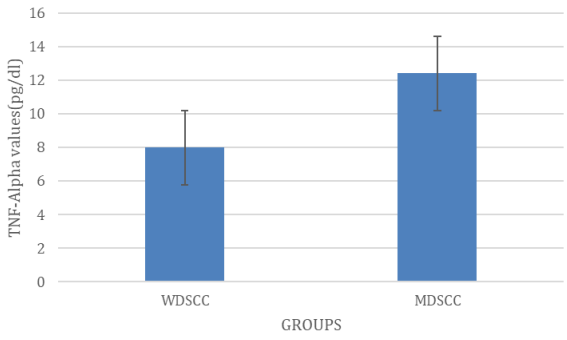


**Fig. 1.** Bar graph showing salivary TNF-α levels in WDSCC and MDSCC. The X-axis represents the type of squamous cell carcinoma and Y-axis represents concentrations of salivary TNF-α.

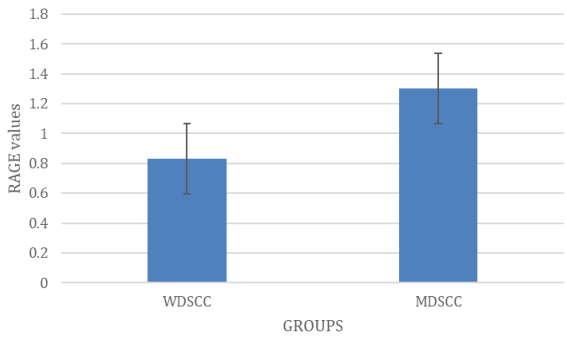


**Fig. 2.** The bar chart shows the median concentration of RAGE in WDSCC samples was 0.83 pg/dl and in MDSCC was 1.3 pg/dl. The graph indicates that there is a significant increase in the salivary levels of RAGE in MDSCC when compared to WDSCC ($p<0.05$).

## Discussion

According to our study, the majority of OSCC patients (50%) are between the ages of 41-50 years, 30% are between the ages of 51-60 years, and 20% are between the ages of 30- 40 years. It was found that the male study population (70%) was higher than the female study population (30%). On comparing the TNF-α and RAGE (Ghrelin) levels in saliva of the patients with squamous cell carcinoma, it was found that both TNF-α and RAGE (Ghrelin) were induced by radiation at high levels with increasing the stage of OSCC. The difference was found to be significant at $p<0.05$.

Salivary TNF-α is a proinflammatory cytokine that is released by macrophages. This type of pro-inflammatory cytokine has a special role in the regulation of the immune response and has a prognostic significance. Enhanced expression of salivary TNF-α has been found in patients with rheumatoid arthritis, chain smokers, and chronic obstructive pulmonary disease.[11] And also, most of the salivary cytokines are majorly correlated with the periodontal status and oral inflammatory issues in recent days.[24] On the other hand, neoplastic lesions also influence salivary inflammatory cytokine in their advanced stages. Mostly the anti-tumor therapy may lead to changes like tumor lysis and that leads to an increase in the salivary cytokines levels.[25]

Salivary diagnostics is a dynamic and emerging research field of molecular technology and diagnostics to aid in the diagnosis of oral and other systemic diseases. Saliva sample collection is easily done and is stored for the early detection of diseases as it contains specific biological markers.[23,26] These days, the saliva sample collection is majorly preferred as it is easily taken without any invasive procedures, and the need for a blood draw would become unnecessary. TNF-α also activates the transcription of NF-κB factor, which helps in the stimulation of cell proliferation, and also mainly increases the secretion of inflammatory cytokines.[27] TNF-α is usually not detectable in normal healthy individuals but increases in the serum and tissue levels are found in inflammatory and infectious conditions and the serum levels well correlate with the severity of the infection. A recent survey done, reported that the medical professionals accepted that screening for medical conditions is important and they were ready to participate when the sample collection was saliva rather than a finger price.[28-30]

In recent days, higher levels of baseline TNF-α is associated with more aggressive behavior of disease and poor survival in patients with immunotherapy resistance.[31] A lower TNF-α in saliva may be related to significantly higher TNF-α levels in serum in a similar group of acute lymphoblastic leukemia children. In the present study, the salivary TNF-α levels were induced at higher rate due to radiation in the case of MDSCC. In contrast to the present study, the study conducted by Deepti G et al., the author concludes that the salivary TNF-α levels were majorly increased in the oral leukoplakia and OSCC as the OSCC patients were majorly having tobacco/smoking habits.[32] In the previous studies done, there was a significant correlation between IL-lβ and sTNF-RI. The mean values of SCC were also elevated as a whole at a particular concentration.[28] IL-6 and TNF-α were considered to be the most sensitive parameters in the early stages of cancer and may be used as an additional biomarker in oral cancer.[33]

Saliva samples of breast cancer patients concluded that when comparing low-stage samples with high-stage cancerous tissues, our results demonstrated that the expression levels of RAGE, at the mRNA and protein levels, were significantly increased in the high-stage samples compared to the low-stage samples explained that two factors are important in progression: inflammation and angiogenesis and RAGE have a significant role in these processes and they found and concluded that mRNA level of RAGE was significantly higher in cancerous tissues compared with that of normal tissues and they also used immunohistochemistry analysis

to confirm and validate of the increased expression of RAGE.[34,35] A certain article explained that RAGEs help regulate cell migration.[36] Few more articles have explained RAGE overexpression of colon cancer, and also the cancer of the stomach, mouth, and tongue through several characteristics like metastasis, invasion, and angiogenesis.[37]

In the present study, the levels of TNF-α and RAGE (Ghrelin) was induced at higher rate due to radiotherapy in MDSCC. As the sample were collected from the cancer patients who attended regularly for all the cycles of the radiotherapy and the expression of salivary TNF-α and RAGE (Ghrelin) in normal healthy individuals stated in the previous studies as at a non-detectable amount, we had a limited sample size and excluded healthy controls respectively.[23] Also, other limitations include systemic illnesses and their treatments among the study population were not taken into account, i.e, whether they take insulin tablets or shots or any other mode of treatment. With further research and a greater sample size, salivary TNF-α and RAGE (Ghrelin) can be used as very good biomarkers of oral cancer.

## Conclusion

According to our study, the majority of OSCC patients (50%) are between the ages of 41-50 years, 30% are between the ages of 51-60 years, and 20% are between the ages of 30- 40 years. It was found that the male study population (70%) was higher than the female study population (30%). Within the limitations of the present study, Salivary TNF-α and RAGE in patients were considered to be induced by radiotherapy at a higher level in MDSCC when compared to WDSCC. Thus, there is an increase in the induced Salivary TNF-α and RAGE levels by radiotherapy with increase in the histological stages of OSCC. This difference was found to be significant and this study further insists that analysis of salivary TNF-α and RAGE (Ghrelin) is very useful for the prediction of prognosis and recurrence rate among patients treated for OSCC.

## Declarations

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None declared by the authors.

***Author contributions***

Conceptualization, M.P.B. and S.R.S.; Methodology, M.P.B.; Software, M.P.B.; Validation, M.P.B., S.R.S. and N.R.; Formal Analysis, M.P.B.; Investigation, M.P.B.; Resources, M.P.B. and N.R.; Data Curation, M.P.B.; Writing – Original Draft Preparation, M.P.B.; Writing – Review & Editing, M.P.B.; Visualization, M.P.B.; Supervision, M.P.B.; Project Administration, M.P.B.; Funding Acquisition, N.R.

***Conflicts of interest***

None to declare.

***Data availability***

Datasets analyzed during the current study are available from the corresponding author after submitting a reasonable request.

***Ethics approval***

Prior to the initiation of the study, clearance was obtained by the Scientific Review Board with the Ethical approval number IHEC/SDC/BDS/1977/01.

## References

1. Obata K, Yutori H, Yoshida K, Sakamoto Y, Ono K, Ibaragi S. Relationships between squamous cell carcinoma antigen and cytokeratin 19 fragment values and renal function in oral cancer patients. *Int J Oral Maxillofac Surg*. 2022;S0901-5027(22)00353-00358. doi: 10.1016/j.ijom.2022.08.019.
2. Meng Q, Wu F, Li G, et al. Exploring precise medication strategies for OSCC based on single-cell transcriptome analysis from a dynamic perspective. *Cancers (Basel)*. 2022;14(19):4801. doi: 10.3390/cancers14194801.
3. Yuan Y, Sturgis EM, Zhu L, et al. A functional variant at the miRNA binding site inE2F1gene is associated with risk and tumor HPV16 status of oropharynx squamous cell carcinoma. *Mol Carcinog*. 2017;56(3):1100–1106. doi:10.1002/mc.22576
4. Nijakowski K, Gruszczyński D, Kopała D, Surdacka A. Salivary metabolomics for oral squamous cell carcinoma diagnosis: A systematic review. *Metabolites*. 2022;12(4):294. doi: 10.3390/metabo12040294.
5. Hyvärinen E, Savolainen M, Mikkonen JJW, Kullaa AM. Salivary metabolomics for diagnosis and monitoring diseases: Challenges and possibilities. *Metabolites*. 2021;11(9):587.doi:10.3390/metabo11090587
6. Hynne H, Sandås EM, Elgstøen KBP, et al. Saliva metabolomics in dry mouth patients with head and neck cancer or Sjögren's syndrome. *Cells*. 2022;11(3):323. doi:10.3390/cells11030323
7. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. *J Clin Invest* . 1996; 15,97(4):1111–1116. doi:10.1172/JCI118504
8. Josephs SF, Ichim TE, Prince SM, Kesari S. Unleashing endogenous TNF-alpha as a cancer immunotherapeutic. *J Transl Med*. 2018;doi:10.1186/s12967-018-1611-7
9. Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation. *J Inflamm*. 1995;46(1):32–41. Available from: https://www.ncbi.nlm.nih.gov/pubmed/8832970
10. Bessaleli E, Scheinfeld N, Kroumpouzos- G. Squamous cell carcinoma of the cervix arising in a patient on adalimumab-a need for cervical screenings in patients

on tumor necrosis factor inhibitors. *Dermatol Online J*. 2018;24(5). Available from: https://www.ncbi.nlm.nih.gov/pubmed/30142745

11. Zielińska K, Karczmarek-Borowska B, Kwaśniak K, et al. Salivary IL-17A, IL-17F, and TNF- Are Associated with Disease Advancement in Patients with Oral and Oropharyngeal Cancer. *J Immunol Res*. 2020;2020:3928504. doi:10.1155/2020/3928504
12. Sethi G, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. Front Biosci. 2008;13:5094–5107. doi:10.2741/3066
13. Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br J Pharmacol*. 1992;107(3):660–664. doi:10.1111/j.1476-5381.1992.tb14503.x
14. ThamaraiSelvi VT, Brundha MP. Salivaomics-A Review. *European Journal of Molecular & Clinical Medicine*. 2020;7(1):2914–2931. Available from: https://www.ejmcm.com/article_3336_7e4099c5dddc87a6bd92447a5c3cbf5e.pdf
15. Rithanya M, Brundha MP. Molecular immune pathogenesis and diagnosis of COVID-19 - A review. *Int J Curr Res Rev*. 2020;69–73. Available from: https://pesquisa.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/pt/covidwho-1011906
16. Wang Y, Yang J, Huang J, Tian Z. Tumor Necrosis Factor-α Polymorphisms and Cervical Cancer: Evidence from a Meta-Analysis. *Gynecol Obstet Invest*. 2020;85(2):153–158. doi:10.1159/000502955
17. Hofmann MA, Drury S, Fu C, et al. RAGE Mediates a Novel Proinflammatory Axis: A Central Cell Surface Receptor for S100/Calgranulin Polypeptides. *Cell*. 1999;97(7):889–901. Available from: https://www.cell.com/cell/abstract/S0092-8674(00)80801-6
18. Tian J, Avalos AM, Mao SY, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol*. 2007;8(5):487–496. doi:10.1038/ni1457
19. Orlova VV, Choi EY, Xie C, et al. A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. *EMBO J*. 2007;26(4):1129–1139. doi:10.1038/sj.emboj.7601552
20. Taguchi A, Blood DC, del Toro G, et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature*. 2000;405(6784):354–360. doi:10.1038/35012626
21. Ishihara K, Tsutsumi K, Kawane S, Nakajima M, Kasaoka T. The receptor for advanced glycation end-products (RAGE) directly binds to ERK by a D-domain-like docking site. *FEBS Lett*. 2003 ;550(1–3):107–113. doi:10.1016/s0014-5793(03)00846-9
22. Syed DN, Aljohani A, Waseem D, Mukhtar H. Ousting RAGE in melanoma: a viable therapeutic target? *Semin Cancer Biol*. 2018;49:20–28. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5910174/
23. Chiamulera MMA, Zancan CB, Remor AP, Cordeiro MF, Gleber-Netto FO, Baptistella AR. Salivary cytokines as biomarkers of oral cancer: a systematic review and meta-analysis. *BMC Cancer*. 2021;21(1):205.doi:10.1186/s12885-021-07932-3
24. Gupta DS, Gupta S, Gupta O, Chandra S. Genetic polymorphism of tumor necrosis factor alpha (TNF-A) and tumor necrosis factor beta (TNF-B) genes and risk of oral pre cancer and cancer. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*. 2019;128(1) p. e88.doi:10.1016/j.oooo.2019.02.224
25. Agnihotri SK, Kumar B, Jain A, et al. Clinical significance of circulating serum levels of sCD95 and TNF-α in cytoprotection of cervical cancer. *Rep Biochem Mol Biol*. 2022;10(4):711–721. doi:10.52547/rbmb.10.4.711
26. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of Salivary Biomarkers in Oral Cancer Detection. *Adv Clin Chem*. 2018;86:23–70. doi:10.1016/bs.acc.2018.05.002
27. Rao E, Hou Y, Huang X, et al. All-trans retinoic acid overcomes solid tumor radioresistance by inducing inflammatory macrophages. *Sci Immunol*. 2021;6(60). doi:10.1126/sciimmunol.aba8426
28. Bossi P, Bergamini C, Miceli R, et al. Salivary Cytokine Levels and Oral Mucositis in Head and Neck Cancer Patients Treated With Chemotherapy and Radiation Therapy. *Int J Radiat Oncol Biol Phys*. 2016;96(5):959–966. doi:10.1016/j.ijrobp.2016.08.047
29. Harsha L, Brundha MP. Prevalence of Dental Developmental Anomalies among Men and Women and its Psychological Effect in a Given Population. *Journal of Pharmaceutical Sciences and Research;* Cuddalore. 2017;9(6):869–873. Available from: https://www.proquest.com/scholarly-journals/prevalence-dental-developmental-anomalies-among/docview/1917938864/se-2
30. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine-A Literature Review. *Research Journal of Pharmacy and Technology*. 2019;12(8):4052–4056. Available from: https://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=12&issue=8&article=088
31. Oton-Leite AF, Silva GBL, Morais MO, et al. Effect of low-level laser therapy on chemoradiotherapy-induced oral mucositis and salivary inflammatory mediators in head and neck cancer patients. *Lasers Surg Med*. 2015;47(4):296–305. doi:10.1002/lsm.22349
32. G D, Nandan SRK, Kulkarni PG. Salivary Tumour Necrosis Factor-α as a Biomarker in Oral Leukoplakia and Oral Squamous Cell Carcinoma. *Asian Pac J Cancer Prev*. 2019;20(7):2087–2093. doi:10.31557/APJCP.2019.20.7.2087
33. Krishnan R, Thayalan DK, Padmanaban R, Ramadas R, Annasamy RK, Anandan N. Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating

premalignant and malignant oral disease. *Asian Pac J Cancer Prev*. 2014;15(17):7141–7148. doi:10.7314/apjcp.2014.15.17.7141

34. Nankali M, Karimi J, Goodarzi MT, et al. Increased Expression of the Receptor for Advanced Glycation End--Products (RAGE) Is Associated with Advanced Breast Cancer Stage. *Oncol Res Treat*. 2016;39(10):622–628. doi:10.1159/000449326
35. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in Inflammation and Cancer, *Annual Review of Immunology*. 2010;28(2):367–388. doi:10.1146/annurev.immunol.021908.132603
36. Bhawal UK, Ozaki Y, Nishimura M, et al. Association of Expression of Receptor for Advanced Glycation End Products and Invasive Activity of Oral Squamous Cell Carcinoma. *Oncology*. 2005;69(3)255. doi:10.1159/000087910
37. Abe R, Yamagishi SI. AGE-RAGE system and carcinogenesis. *Curr Pharm Des*. 2008;14(10):940–945. doi:10.2174/138161208784139765