Comparison of Matrix Metalloproteinases 2 and 9 Levels in Saliva and Serum of Patients with Head and Neck Squamous Cell Carcinoma and Healthy Subjects

Zohreh Dalirsani 1, Atessa Pakfetrat 1, Zahra Delavarian 1, Seyed Isaac Hashemy 2, Leila Vazifeh Mostaan 3, Marzieh Abdollahnejad 4, Azar Fani Pakdel 5, Elham Banihashemi 4 and Ala Ghazi 1, *

1Oral and Maxillofacial Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4Dentist, Mashhad, Iran
5Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding author: Oral and Maxillofacial Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Email: alaghazi10@gmail.com

Received 2019 February 04; Revised 2019 April 22; Accepted 2019 April 24.

Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth common cancer worldwide. A hallmark of cancer progression is degradation of the extracellular matrix by matrix metalloproteinases (MMPs) that allows cancer cells to invade the surrounding tissue.

Objectives: The purpose of this study was to evaluate the levels of MMP-2 and 9 in serum and salivary of HNSCC patients and compare it with a healthy group.

Methods: Twenty patients with newly diagnosed HNSCC, who had not received any treatment, referred to Omid and Ghaem hospitals, Mashhad, Iran, and twenty healthy controls were voluntarily included in this study. Salivary and blood samples were collected from both groups and the concentration of MMP-2 and MMP-9 were measured by enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed by SPSS using Student t-test or Mann-Whitney with significance level of ≤ 0.05.

Results: In cancer patients, the serum level of MMP-9 was significantly higher than that of in the healthy group (P < 0.001). However, salivary MMP-9 was higher in cancer patients, this difference was not significant (P = 0.736). There was no significant difference between the study groups for the levels of serum (P = 0.283) and salivary MMP-2 (P = 0.764). There was a correlation between salivary and serum levels of both markers in cancer patients (P = 0.046 and P = 0.011 for MMP-2 and MMP-9, respectively), on the contrary, there was not a correlation between them in the healthy controls (P = 0.628 and P = 0.064, for MMP-2 and MMP-9, respectively). A direct correlation between the salivary level of MMP-9 and tumor grade was also detected (P = 0.045).

Conclusions: The salivary analysis indicates that an altered composition for MMPs in HNSCC, suggesting a potential diagnostic tool for oral cancer. The serum level of MMP-9 appears to be a reliable marker for early diagnosis in HNSCC patients.

Keywords: Head and Neck Squamous Cell Carcinoma, Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, Saliva, Serum

1. Background

Squamous cell carcinoma accounts for more than 90% of head and neck cancers (1). Despite many advances have been made in recent decades in therapeutic fields such as surgery, chemotherapy, and radiotherapy, the 5-year survival rate is still lagging behind (2). The advanced lesions of oral squamous cell carcinoma (OSCC) often occur with pain, halitosis, difficulties in speaking, and swallow, whereas the initial lesions do not have any symptoms (3). Although the oral cavity can be directly examined, most patients are detected with weak prognosis in the advanced stages of the disease (4). It seems that delay in diagnosis is the primary reason for high mortality rates associated with this cancer (5). Therefore, an early diagnosis would lead to better treatment results and can increase the five-year survival rate up to about 80% (6).

The lack of early warning signs represents the importance of finding biomarkers with high sensitivity and specificity for early detection and screening of high-risk individuals (2). Matrix metalloproteinases (MMPs) are a family of extracellular zinc-dependent endopeptidases capable of selectively degrading various components of the extracellular matrix (ECM) and non-matrix proteins. Most
of the members of this family share a catalytic domain with a conserved zinc binding site followed by a C-terminal hemopexin-like domain involved in the recognition of specific substrates. MMP-2 (gelatinase A) and MMP-9 (gelatinase B), which harbor an additional collagen-binding domain inserted in the catalytic domain that binds collagens and fibronectin. MMPs are upregulated in various types of human cancer and are associated with tumor progression including invasion, angiogenesis and metastasis, and poor prognosis. MMP-2 and MMP-9 are the most studied MMPs in cancer since their overexpression in tumor cells is linked to metastasis and advanced tumor stages. Recent investigations have shown that these 2 gelatinases play important roles in migration, invasion, and angiogenesis of tumor cells by releasing ECM-associated growth factors, modulating the activities of cytokines and chemokines, and inducing critical intracellular signalling cascades through interaction of their hemopexin (PEX) domain with cell surface receptors (7).

2. Objectives

To date, there is no study on the evaluation of serum and salivary levels of MMP-2 and MMP-9 in patients with OSCC. In this study, the protein levels of MMP-2 and MMP-9 in the saliva and serum of patients with head and neck SCC (HNSCC) were compared with those of the healthy subjects in a case-control study and their relationships with the stage and grade of SCC were determined. In addition, the salivary and serum levels of these proteins were evaluated within each group to determine whether there is a direct correlation between their serum and salivary levels.

3. Methods

In this pilot case-control study, 20 patients with head and neck squamous cell carcinoma presented to the Omid and Qaem hospitals in Mashhad, Iran from October 2014 to June 2015, were selected using purposive non-probability sampling technique. HNSCC of the case group was confirmed by histopathology and they had not received any treatment before the sampling. The control group was included 20 normal volunteers who had no history of malignancy or immunodeficiency.

Patients with immune deficiency diseases, other malignancies, renal or liver diseases were not included in the study. Additional exclusion criteria were the presence of any kind of oral malignancy in the control group during the experiment and also the patient’s dissatisfaction.

Demographic data such as age and gender; malignancy-related features including the site of involvement, symptoms and TNM (T: primary tumor size; N: regional lymph nodes involvement; M: metastasis) as well as the history of alcohol and smoking consumption were recorded in special forms based on the medical records and interviews with the patients.

3.1. Saliva Sampling

Saliva sampling was performed according to the standard technique. At 9 to 11 A.M., participants were asked not to eat, drink, smoke or use any hygienic device during at least an hour before sample collection. To prevent the saliva proteins from degradation, the samples were stored at -80°C until being analyzed (2). The saliva samples were centrifuged at 4500 g for 20 minutes to separate the cells and debris.

3.2. Serum Sampling

Blood samples were drawn before treatment. Serum was separated within an hour after blood collection; the venous blood samples were kept at room temperature for 30 minutes to be clotted, and then were centrifuged at 2000 rpm for 15 minutes. Afterwards, sera were collected and kept at -80°C until being analyzed.

3.3. Analysis of Serum and Salivary MMP-2 and 9 Levels by Sandwich ELISA Technique

MMP-2 and MMP-9 protein concentrations in all samples were measured by Human MMP-9 Quantikine ELISA Kit (R&D Systems #DMP900, USA) and Human MMP-2 Quantikine ELISA kit (R&D Systems #DMP2F0, USA) according to their manufacture’s instructions.

The data were presented in nanograms per mL. In this experiment, serum and salivary levels of both active type (82 kD), as well as the pre-enzyme (92 Dk) of MMP-9 protein was measured in a 3.5-hour phase assay; and MMP-2 level was quantified in a 4.5-hour ELISA solid phase assay. Briefly, the microtiter plates were coated with either anti-MMP-2 or anti-MMP-9 polyclonal antibodies, and the standard solutions and samples were transferred into the wells. After washing the unbound materials, the enzyme-linked polyclonal antibody against either MMP-2 or MMP-9 antibodies was added. This was followed by another washing step and the addition of the substrate solution. The developed color was fixed and the light absorbance at 450 nm was measured by a micro-plate reader.

3.4. Ethical Considerations

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran. The methodology was described to all patients and the informed consent was obtained from the study participants.
3.5. Statistical Analysis

In order to data collection, data were analyzed by SPSS (version 16) software. The descriptive analysis was done by using tables and charts, while the explanatory analysis was conducted using the 2 appropriate mean comparison test (based on the normality or non-normality of the data, the t-test or Man Whitney test was used, respectively) and the Spearman correlation test. The significance level was set at ≤ 0.05 for all tests.

The assessment of the relationship between the markers and metastases, grade, location, and age was done with the independent t-test, while the relationship of the markers with the stage of the disease and correlation between the serum and salivary levels of each protein was assessed with the Pearson test.

4. Results

A total of 20 HNSCCs were identified with 9 male and 11 female patients, with the average age of 60.95 ± 15.32 years. The control group was comprised of 20 normal volunteers including 5 women and 15 men, with the mean age of 53.30 ± 4.48 years.

The tumor site was intra-oral in 11 participants (tongue, Alveolar ridge, palate) and extra-oral cavity in 9 patients (larynx, hypopharynx, and nasopharynx). Stages and grades of the tumors are shown in Table 1. Eleven patients had metastases to lymph nodes and 9 patients had no metastasis.

| Grade | I | II | III | IV |
|-------|---|----|-----|----|
| Stage | 2 | 6  | 5   | 7  |

Table 1. Number of Patients According to Grade and Stage of Tumor

Statistically, a significant difference was not observed in the salivary levels of MMP-2 in the control (2.21 ± 0.31 ng/mL) and case (2.17 ± 0.62 ng/mL) groups (P = 0.764). Although the serum levels of MMP-2 in the case group (12.48 ± 2.19 ng/mL) was higher than that in the control group (11.69 ± 2.39), but this difference was not statistically significant (P = 0.283) (Table 2).

The salivary levels of MMP-9 in the case group (49.27 ± 44.5 ng/mL) were higher than the control group (44.68 ± 40.95 ng/mL), but this difference was also not statistically significant (P = 0.736) (Table 2). In contrast, the serum levels of MMP-9 in the case group (78.77 ± 27.51 ng/mL) was substantially higher than that of in the control group (39.89 ± 20.54 ng/mL) with a statistically significant difference (P < 0.001) (Figure 1).

Table 2. Levels of MMP-2 and 9 in the Serum and Saliva of the Case and Control Group*

| Variables      | Study Groups, ng/mL | P Value |
|----------------|---------------------|---------|
|                | Case                | Control |
| MMP-2 saliva   | 2.17 ± 0.62         | 2.21 ± 0.31 | 0.764 |
| MMP-2 serum    | 12.48 ± 2.19        | 11.69 ± 2.39 | 0.283 |
| MMP-9 saliva   | 49.27 ± 44.50       | 44.68 ± 40.95 | 0.736 |
| MMP-9 serum    | 78.77 ± 27.51       | 39.89 ± 20.54 | < 0.001 |

Abbreviation: MMP, matrix metalloproteinase.

Values are expressed as mean ± SD.

Pearson test in the case group revealed a significant direct relationship between serum and salivary levels of MMP-2, and also between serum and salivary levels of MMP-9 (r = 0.452, P = 0.046) and (r = 0.558, P = 0.011), respectively; while no significant relationship was observed in the control group [(r = -0.115, P = 0.628) and (r = 0.422, P = 0.064), respectively] (Table 3).

Moreover, the tumor site was subdivided into intraoral or extraoral cavity to evaluate the relationship between variables and tumor site. The results of T independent test demonstrated that serum and salivary levels of MMP-2 or 9 did not have a significant relation with tumor site and metastasis.

Regarding the histopathologic grade of tumors, t-test revealed that there was no significant relation between serum levels of MMP-2 or 9 as well as salivary levels of MMP-2 with the grade of the disease; whereas the relation between salivary levels of MMP-9 and the tumor grade was statistically significant (P = 0.045) (Figure 2).

In addition, the results of ANOVA and Spearman tests...
Table 3. Comparison of Levels of MMP-2, and 9 in the Serum and Saliva of the Case and Control Group

| Groups/Variables | Type of Sample | Double t-test | Pearson Correlation Coefficient | P Value |
|------------------|----------------|--------------|----------------------------------|---------|
|                  |                |              |                                  |         |
| Case             |                |              |                                  |         |
| MMP-2            | Saliva         | 2.17 ± 0.62  | 12.48 ± 2.19                     | > 0.001 | 0.452 | 0.046 |
| MMP-2            | Serum          | 12.48 ± 2.19 |                                    |         |
| MMP-9            | Saliva         | 49.27 ± 44.50| 78.77 ± 27.51                    | 0.002   | 0.558 | 0.011 |
| MMP-9            | Serum          | 78.77 ± 27.51|                                    |         |
| Control          |                |              |                                  |         |
| MMP-2            | Saliva         | 2.21 ± 0.31  | 11.69 ± 2.39                     | > 0.001 | -0.115 | 0.628 |
| MMP-2            | Serum          | 11.69 ± 2.39 |                                    |         |
| MMP-9            | Saliva         | 44.68 ± 40.95| 39.89 ± 20.54                    | 0.572   | 0.422 | 0.064 |
| MMP-9            | Serum          | 39.89 ± 20.54|                                    |         |

Abbreviation: MMP, matrix metalloproteinase.

Figure 2. Analysis of the relation between salivary levels of MMP9 and the grade of tumor demonstrated that there was no significant relationship between serum and salivary levels of MMP-2 or 9 with tumor stage.

As presented in Tables 2 and 4, the serum levels of MMP-9 showed no correlation with clinical parameters such as the tumor location, clinical stage, histopathological grade, and lymph node metastasis. Interestingly, the salivary levels of MMP-9 were significantly associated with the tumor histopathological grade (Table 4). No correlation was found between salivary or serum levels of MMP-2 and any of the clinical parameters.

5. Discussion

Recent achievements in the field of biological research have provided a better understanding of the molecular processes involved in the pathogenesis and progression of HNSCC and led to the identification of a large number of biomarkers. However, further studies are needed in order to make the clinical application of these biomarkers possible.

MMP-2 and 9 are proteases which play a role in removing the extracellular matrix through collagen IV decomposition and are thus involved in the invasion of tumors and metastasis.

MMP-9 plays a role in inflammation, wound healing, tissue remodeling, movement of matrix-bonded growth factors, and cytokine’s processing (8). MMP-2 (gelatinase A) is expressed at high levels during the growth. This enzyme increases at the sites of tissue damage, inflammation and in the stromal cells of the invading edge of the metastatic tumors.

High expression levels of MMP-9 and MMP-2 was shown in different cancers including HNSCC (9-11). Most of these studies have focused on tissue samples and showed an increased expression of these proteins in tumoral tissues. While, most published research in the field of cancer biomarkers have focused on blood components such as serum, plasma, and urine. Saliva as a source of biomarkers has captured less attention (12). In addition, advantages of the salivary sample can be noted due to the ease of collection, no need for special equipment and a trained person for sampling, and non-invasive and simplicity nature of the sample collection. Thus the establishment of the salivary diagnostic methods in these patients is highly valued (5).

According to the results of the present study, the mean serum levels of MMP-9 in patients with HNSCC were considerably higher than normal subjects. Further, serum and salivary levels of either MMP2 or 9 in the case group were significantly correlated; but this correlation was not observed in the control group.

In the study conducted by Shpitzer et al. (13) MMP-2 and MMP-9 proteins were measured in salivary samples of OSCC patients in which the tumor location was in the lateral border of the tongue. The salivary concentration of
Table 4. Serum and Salivary Levels of MMP-2 and 9 in SCC Patients in Relation to the Clinicopathological Features of Tumor

| Tumor Site | Grade | Stage | Metastasis |
|------------|-------|-------|------------|
|            |       |       |            | P Value  | Correlation Coefficient |
| Salivary MMP-2 | 0.717 | 0.373 | 0.217 | 0.289 | 0.377 |
| Serum MMP-2  | 0.735 | 0.539 | 0.265 | 0.262 | 0.249 |
| Salivary MMP-9 | 0.677 | 0.045 | 0.414 | -0.193 | 0.874 |
| Serum MMP-9  | 0.772 | 0.250 | 0.759 | -0.073 | 0.977 |

Abbreviation: MMP, matrix metalloproteinase.

MMP-2 and MMP-9 in the case group were higher than the control group. However, in the present study, although the salivary levels of MMP-9 were higher in case subjects, the difference was not significant. In another study in which the MMP-9 concentration was measured in the salivary samples of patients with tongue SCC, its concentration was considerably higher in the patients in comparison with the control subjects (14).

Regarding the serum levels of these proteins in studies of Wang et al. (15), Cheng et al. (16), and Ranuncolo et al. (17), the MMP-9 concentration was reported to be significantly higher in patients with HNSCC compared to the control group. These results were similar to the results of the present study in which serum concentration was significantly lower in healthy subjects than SCC patients.

In the other studies (18-22), the MMP-9 protein was represented as a better marker compared with MMP-2 for the evaluation of metastasis and malignant changes and for assessing the clinical features, the prognosis predicting factor, and the accurate grading of tumors. Moreover, in Patel et al. (23, 24) and Xu et al.’s (25) studies, the incidence of MMP-2 was associated with lymph node metastases. The disagreement between these studies might be the result of the variety in tumor locations, the number of samples, population differences, and the diversity in sensitivity of the applied techniques.

As it has been observed in several studies, the serum levels of MMP-9 in patients with HNSCC are significantly higher than that of healthy and treated patients, but this difference was not observed for MMP-2 levels, which is consistent with the results of the present study. Therefore, although there are limited studies in this area, it seems that MMP-2 is not a reliable marker for diagnosis and prognosis of HNSCC.

Due to the limitations of the present study such as small sample size, high diversity in tumors locations, and the lack of age/sex matching between the 2 groups, we suggest that for future studies, researchers use a larger population in which these parameters are matched.

The aim of this study was to evaluate the levels of MMP-2 and 9 in serum and salivary of HNSCC patients and compare them with a healthy group. Consistently with previous studies, the results of this study demonstrated that the serum levels of MMP-9 were considerably higher in patients compared with the control group. Although the levels of MMP-9 in salivary were higher in the case group, it showed no significant difference with the control group; however, it was correlated with the tumor histopathological grade. Furthermore, the salivary levels of this proteinase significantly correlate with the serum levels in the case group. In the present study, the serum and salivary levels of MMP-2 were not significantly different between case and control group. However, there was a significant correlation between its serum and salivary levels in the case group.

Since previous studies have also found the high levels of MMP-9 marker in the patients’ serum, it appears that if this conclusion would be proved in further studies, the serum levels of MMP-9 can be a reliable marker for diagnosis and analysis of the response to treatment in patients with HNSCC. Given the scarcity of the studies on the serum and salivary levels of MMP-9 and MMP-2 in patients with HNSCC, more extensive studies are needed in future.

Acknowledgments

It is not declared by the authors.

Footnotes

Authors’ Contribution: Authors participated in the revision.

Conflict of Interests: The authors declare no conflict of interest in this study.

Ethical Considerations: The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran.

Financial Disclosure: It is not declared by the authors.

Funding/Support: The vice-chancellor for Research at Mashhad University of Medical Sciences.
References

1. Gregoire V, Lefebvre JL, Licitra L, Felip E; EHNS-ESMO-ESTRO Guidelines Working Group. Squamous cell carcinoma of the head and neck: EHNS-ESMO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2010;21 Suppl 3:S184–6. doi: 10.1093/annonc/mdq085. [PubMed: 20555077].

2. Saheb Jamee M, Eslami M, Atarbash Moghadam F, Sarafnejad A. Salivary concentration of TNalpha, IL1 alpha, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008;13(5):E292-5. [PubMed: 18449112].

3. Stich HF, Mathew B, Sankaranarayanan R, Nair MK. Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of beta-carotene or vitamin A. Am J Clin Nutr. 1993;58(3 Suppl):2985-3045. doi: 10.1093/ajcn/58.3.2985. [PubMed: 1985402].

4. Streekofs CF, Dubinsky WP. Proteomic analysis of saliva for cancer diagnosis. Expert Rev Proteomics. 2007;4(3):329-32. doi: 10.1586/14789450.4.3.329. [PubMed: 17552931].

5. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Res Clin Cancer Res. 2008;14(9):5246-52. doi: 10.1038/0047-0342.CCR-07-5037. [PubMed: 18829504]. [PubMed Central: PMC2877125].

6. de Jong EP, Xie H, Oonsong G, Stone MD, Chen XB, Kooren JA, et al. Quantitative proteomics reveals myosin and actin as promising saliva biomarkers for distinguishing pre-malignant and malignant oral lesions. PLoS One. 2010;5(6). e1148. doi: 10.1371/journal.pone.0011148. [PubMed: 20567502]. [PubMed Central: PMC2887353].

7. Mroczko B, Lukaszewicz-Zajac M, Gryko M, Kedra B, Szmitkowski M. Clinical significance of serum levels of matrix metalloproteinase 2 (MMP-2) and its tissue inhibitor (TIMP-2) in gastric cancer. Folia Histochem Cytobiol. 2004;42(1):25-31. doi: 10.5603/FHC.2004.0018. [PubMed: 15264949].

8. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, et al. Matrix metalloproteinases: A review. Crit Rev Oral Biol Med. 1993;4(2):197-250. doi: 10.1077/1045-4490(1993)004[0197]250[0400]. [PubMed: 8435466].

9. Rosenhal EL, Matrisian LM. Matrix metalloproteases in head and neck cancer. Head Neck. 2006;28(7):639-48. doi: 10.1002/hed.20165. [PubMed: 16470875]. [PubMed Central: PMC2873217].

10. Pramanik KK, Nagini S, Singh AK, Mishra P, Kashyap T, Nath N, et al. Glycogen synthase kinase-beta mediated regulation of matrix metalloproteinase-9 and its involvement in oral squamous cell carcinoma progression and invasion. Cell Oncol (Dordr). 2018;41(1):47-60. doi: 10.1007/s10434-017-0358-0. [PubMed: 29134466].

11. Jose D, Mane DR. Correlation of matrix metalloproteinase-9 expression with morphometric analysis of mucosal vasculature in oral squamous cell carcinoma, oral epithelial dysplasia, and normal oral mucosa. J Int J Health Sci (Qassim). 2018;12(6):36-43. [PubMed: 30534042]. [PubMed Central: PMC6257875].

12. Dowling P, Wormald R, Meleady P, Henry M, Curran A, Clynes M. Analysis of the saliva proteome from patients with head and neck squamous cell carcinoma reveals differences in abundance levels of proteins associated with tumour progression and metastasis. J Proteomics. 2008;71(2):468-75. doi: 10.1016/j.jprot.2008.04.004. [PubMed: 1841744].

13. Shpitzer T, Bahar G, Feinmesser R, Nagler RM. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol. 2007;133(9):513-7. doi: 10.1007/s00432-007-0207-2. [PubMed: 17479291].

14. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borrovi I, et al. Salivary analysis of oral cancer biomarkers. Br J Cancer. 2009;100(7):1194-8. doi: 10.1038/bjc.2009.290. [PubMed: 1978935]. [PubMed Central: PMC2768098].

15. Wang WL, Chang WL, Yeh YC, Lee CT, Chang CY, Lin JT, et al. Concomitantly elevated serum matrix metalloproteinases 3 and 9 can predict survival of synchronous squamous cell carcinoma of the upper aerodigestive tract. Mol Cancer. 2013;12(6):438-45. doi: 10.1002/mc.22874. [PubMed: 22241750].

16. Cheng D, Kong H, Li Y. Prognostic value of interleukin-8 and MMP-9 in nasopharyngeal carcinoma. Eur Arch Otorhinolaryngol. 2014;271(3):503-9. doi: 10.1007/s00405-013-2580-3. [PubMed: 23749058].

17. Ranuncolo SM, Matos E, Loria D, Vilenksy L, Rojo R, Bal de Kier Joffe E, et al. Circulating 92-kilodalton matrix metalloproteinase (MMP-9) activity is enhanced in the egulobulin plasma fraction of head and neck squamous cell carcinoma. Cancer. 2002;94(5):1483-91. doi: 10.1002/cncr.10356. [PubMed: 11920505].

18. Hong SD, Hong SP, Lee JI, Lim CY. Expression of matrix metalloproteinase-2 and -9 in oral squamous cell carcinomas with regard to the metastatic potential. Oral Oncol. 2000;36(2):207-13. doi: 10.1006/jore.1999.0375. [PubMed: 10745754].

19. Cao XI, Xu RJ, Zheng YY, Liu J, Teng YS, Li Y, et al. Expression of type IV collagen, metalloproteinase-2, -9, and tissue inhibitor of metalloproteinase-1 in laryngeal squamous cell carcinomas. Asian Pac J Cancer Prev. 2011;12(12):3245-9. doi: 22476416.

20. Lee SY, Park SY, Kim SH, Choi EC. Expression of matrix metalloproteinases and their inhibitors in squamous cell carcinoma of the tonsil and their clinical significance. Clin Exp Otorhinolaryngol. 2011;4(2):88-94. doi: 10.3342/ceo.2011.4.2.88. [PubMed: 2716955]. [PubMed Central: PMC3109333].

21. Jordan RC, Macabeo-Ong M, Shiboski CH, Dekker N, Ginzinger DG, Wong DT, et al. Overexpression of matrix metalloproteinase-1 and -9 mRNA is associated with progression of oral dysplasia to cancer. Clin Cancer Res. 2004;10(9):3840-5. doi: 10.1158/1078-0432.CCR-04-0656. [PubMed: 1547543].

22. Mohlizahn N, Babakoohi S, Shiva A, Shadman A, Kamayeb-Hesari K, Shakeri MT, et al. Immunohistochemical study of PS1, PS2, MMP-2 and MMP-9 expression at invasive front of squamous cell and verrucous carcinoma in oral cavity. Pathol Res Pract. 2013;209(2):310-4. doi: 10.1016/j.prp.2012.11.002. [PubMed: 21279944].

23. Patel BP, Shah SV, Shakul SN, Shah PM, Patel PS. Clinical significance of MMP-2 and MMP-9 expression at invasive front of squamous cell and verrucous carcinoma in oral cavity. J Surg Oncol. 2005;90(2):81-8. doi: 10.1002/jso.20240. [PubMed: 15844188].

24. Patel BP, Shah SV, Shakul SN, Shah PM, Patel PS. Clinical significance of MMP-2 and MMP-9 in patients with oral squamous cell carcinoma. J Surg Oncol. 2005;90(2):81-8. doi: 10.1002/jso.20240. [PubMed: 15844188].

25. Xu YP, Zhao XQ, Sommer K, Moubayed P. Correlation of matrix metalloproteinase-2, -9, tissue inhibitor-1 of matrix metalloproteinase and CD44 variant 6 in head and neck cancer metastasis. J Zhejiang University Sci B. 2003;4(4):491-501. doi: 10.1016/S1673-5428(03)40941. [PubMed: 12881629].

Dalirani Z et al.