Short Communication
ISSN 2413-0516

Is salivary evaluation of P53 and MMP-3 a good tool for early detection of oral squamous cell carcinoma?

Shima Nafarzadeh,[a] Mohsen Emamgholipour,[b] Fatimah Bijani,[c] Hamed Hosseinkazemi,[a] Amrollah Mostafazadeh,[d] Oveis Khakbaz,[a] Fatemeh Baladi,[d] and Soraya Khafri[e]

[a]Oral Health Research Center, Institute of Health, Babol University of Medical Sciences, Babol, Iran.
[b]Student Research Committee, Babol University of Medical Sciences, Babol, Iran.
[c]Department of Oral and Maxillofacial Pathology, Dentistry School, Babol University of Medical Sciences, Babol, Iran.
[d]Babol University of Medical Sciences, Babol, Iran.
[e]Department of Biostatistics and Epidemiology, Faculty Member of Biostatistics Sciences, Babol University of Medical Sciences, Babol, Iran.
Correspondence to Mohsen Emamgholipour (email:mohsenemamgholipour@gmail.com).
(*Submitted: 24 June 2018 – Revised version received: 02 July 2018 – Accepted: 05 August 2018 – Published online: 26 September 2018*)

Objective Oral squamous cell carcinoma (OSCC) is one of the most common malignancies around the world. Despite the advancement in treatment methods, the prognosis is still not good. Based on clinicians' idea, the early diagnosis of the lesion can lead to better prognosis. Some salivary biomarkers such as matrix metalloproteinase-3 and P53 may detect OSCC in early stages. In this study, we wanted to compare salivary MMP-3 and P53 levels in OSCC patients and control group.
Methods Fifteen patients with OSCC (9 male and 6 female) were selected from Oral Pathology Department, Babol, Iran. Salivary MMP-3 and P53 were measured by ELISA and compared with control group. Data was analyzed by ANOVA, *t*-test, and Mann–Whitney.
Result There was no significant differences between salivary MMP-3 and P53 concentration in patients with OSCC and healthy individuals.
Conclusion Based on our findings and other similar studies, salivary MMP-3 and P53 levels might not be accurate enough to detect early stages of OSCC. But there are controversial statements about these questions. So, supplementary studies are needed to be done in future.
Keywords OSCC, saliva, P53, MMP3, ELISA9

Introduction

Head and neck cancer including oral cavity, pharynx, hypopharynx, and larynx is one of the most common malignancies around the world.[1] Moreover, 90% of head and neck cancers have been diagnosed as squamous cell carcinoma (SCC).[2] Cancer is a major cause of mortality worldwide and a lot of new cases occur every year.[3]

The process of oral carcinogenesis is multifactorial and multistep, and the exact sequence is almost unknown.[4,5] In spite of presence of risk factors such as tobacco and alcohol in some patients, most of them ultimately are not diagnosed as malignancies. This implies that genetic has an important role in susceptibility to cancers.[6]

Unfortunately, despite the advanced methods in surgery, radiotherapy and chemotherapy, the prognosis of oral SCC (OSCC) is not good yet. But in case of early detection, the prognosis could be better.

Biopsy followed by histopathological evaluation is gold standard for diagnosis of OSCC, but it is more helpful in late stages of disease. Biomarkers are measurable indicators which can be useful for early diagnosis of some lesions.[7] The most common laboratory diagnostic procedures involve the chemical and cellular analysis of blood. Other biologic fluids like saliva are also used in diagnostic tests.[8] Salivary biomarkers in oropharyngeal carcinoma are cost-effective. Saliva contains a wide range of components and is easy access, so patients feel more comfortable. The method is also non-invasive and handling is safe. There are also other advantages for using saliva instead of blood.[7] Therefore, nowadays there is a tendency to use of salivary biomarkers as diagnostic and prognostic factors.

The matrix metalloproteinase (MMP) family involves diverse substrates.[9] They are large family of zinc-dependent endopeptidase, and they have ability to digest extracellular matrix.[10] Generally, MMPs are made up of a prodomain, a catalytic domain, a hinge region, and a hemopexin domain. They are secreted from the cell or anchored to the plasma membrane. MMPs have six separated groups: Collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others.[11]

Matrix metalloproteinase-3 (stromelysin-1) is a secretory enzyme which several growth factors regulate its expression and the process of wound healing can stimulate its secretion.[12] It is expressed by keratinocytes, fibroblasts, and chondrocytes.[13] It has found that overexpression of MMP3 is in association with developing malignancies including head and neck carcinomas.[14,15]

The P53 protein is activated after DNA damage or oncogenic signals. It has an important role in cell cycle control, DNA repair, and apoptosis.[16] Altered expression and functional loss of P53 are common genetic changes in human malignancies.[17,18]

Until now, conflicting results have been obtained from the study on MMP-3 and P53 markers in OSCC patients, which could be due to differences and limitations in the immunohistochemical and RT-PCR methods in semi-quantitative cytokine assessments. In addition, these two markers have not been evaluated simultaneously in patients with OSCC. Therefore, in this study, we decided to use a completely quantitative method to examine the levels of salivary P53 and MMP-3 in OSCC patients to find their real changes in OSCC affected patients. Among the available methods, studies can perform on the tissue blocks, blood, and other biologic fluids. Saliva is believed to be a reliable tool for diagnosis of OSCC because it is in direct contact with cancerous tissue.[7]

This study was designed based on the evaluation of P53 and MMP-3 in whole unstimulated saliva of patients with OSCC using ELISA method.

Materials and Methods

Fifteen patients with OSCC were selected from Oral and Maxillofacial Pathology Department of Dentistry Faculty of Babol University of Medical Sciences, Babol, Iran. A total of 9 patients were male and the rest of them were female. Their age range was between 51 and 89. Their diagnosis were proven by biopsy and histopathological evaluation. Invasion of dysplastic tumoral epithelial cells into the connective tissue was essential for diagnosis. All slides were re-evaluated to confirm the diagnosis. All of them were in grades I and II. We informed the patients and asked them to participate in our study. Oral and written consents were obtained from participants.

Moreover, 30 healthy individuals without any dysplastic or malignant lesions were selected as control group. Systemic diseases, smoking, chronic periodontitis, use of NSAIDs, corticosteroids, and antihistamines, history of chronic lung diseases or any treatment for malignancy, and pregnancy for women were our other exclusion criteria.

Salivary specimens were collected at the morning between 9 and 12 AM. They were banned from eating, drinking, chewing gum, and smoking for at least 2 h before sampling. Saliva samples were obtained in a dry and sterile tube of 50 ml (1–5 ml for each factor). All samples were immediately centrifuged at 4°C for 20 min at a speed of 6000 rpm to separate the cells and then were stored at −80°C until final analysis.

Matrix metalloproteinase-3 and P53 salivary concentration were measured by ELISA according to the manufacturer's instructions (E1711Hu and E0907Hu, Shanghai Crystal Day Biotech Co., China).

T-test and Mann–Whitney test were used for statistical analysis.

Results

About 9 men and 6 women participated in our study with the diagnosis of OSCC. The tongue was the most site of involvement.

T-test showed no significant difference in saliva P53 concentration among control (mean ± SD: 1011.136 ± 655.323) and OSCC (mean ± SD: 709.215 ± 454.961) groups ($P = 0.171$).

Mann–Whitney analysis showed no significant differences in saliva concentration of MMP-3 among control (mean ± SD: 45.406 ± 67.349) and SCC (mean ± SD: 35.706 ± 43.844) groups ($P = 0.268$).

T-test showed no significant differences in saliva P53 concentration among men (mean ± SD: 755.808 ± 499.032) and women (mean ± SD: 639.326 ± 413.945) with SCC ($P = 0.654$).

Mann–Whitney analysis showed no significant differences in saliva concentration of MMP-3 among men (mean ± SD: 45.177 ± 53.002) and women (mean ± SD: 21.499 ± 22.004) with SCC ($P = 0.906$) (Fig. 1).

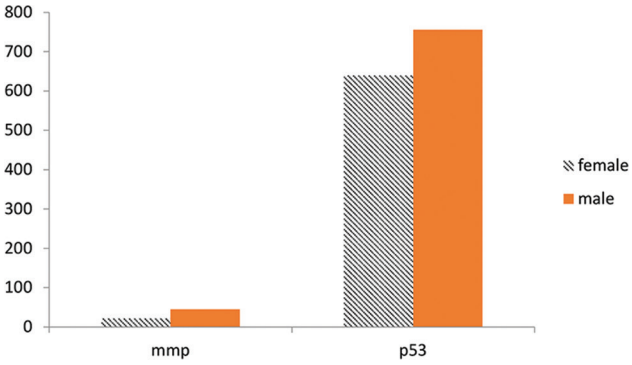

Fig. 1 **Unstimulated whole saliva concentration of P53 and MMP-3 in patients with SCC in men and women. Data are expressed as mean ± SD. $P < 0.05$ is significant.**

Discussion

Oral squamous cell carcinoma is one of the most common malignancies in head and neck region. Despite the advancement in therapeutic techniques, the prognosis has not been improved significantly. To achieve the goal, early diagnosis and treatment is essential. Nowadays, tendency to use biomarkers is increasing. Scientists are in favor with salivary biomarkers because their stimulation is safe and simple.

In recent years, many researches have been done on serum and salivary biomarkers to find diagnostic and prognostic markers in malignancies. In the present research, we compared salivary MMP-3 and P53 levels in OSCC patients and control group.

Zhang et al.[19] in a meta-analysis study on Asian and European societies showed that there was no significant association between MMP-3 level and head and neck cancer risk. In contrast, in European subgroup MMP-3 polymorphism was significantly in association with HNC risk.[19] Our study took place in Iran and our result was the same as Asian subgroup in their research. About these findings we hypothesize that MMP-3 could be a diagnostic marker in some nations like Europeans.

On the other hand, some authors stated in their article that the concentration of salivary MMP-3 level elevated in patients with oral SCC in compared to control group.[20] But our examination did not show significant differences in these groups.

Agha-Hosseini et al.[21] in their study showed that serum and unstimulated salivary levels of MMP-3 are significantly correlated with each other and both of them increase in OSCC patients with higher stages of tumor. We did not work on patients' serum biomarkers but there was no relation between salivary MMP-3 and risk of malignancy. Moreover, all of our patients were in low grade.

Taghavi et al.[22] in an immunohistochemical study on esophageal squamous cell carcinoma have not found a significant association between tumor and adjacent normal tissues based on P53 positive expression, but they stated that there was significant positive expression of P53 in esophageal SCC patients in comparison with healthy population. Other authors also showed that P53 antibody could be detected in the saliva of patients with OSCC and might be useful for early detection and screening.[23] In other experiments overexpression of muted and unmuted P53 in patients with SCC was detected.[24] Some other studies on salivary P53 level showed higher levels in OSCC patients compared to control group.[25,26] Conflicting findings in other research showed that there is still no clear relationship found between P53 overexpression and known risk factors for esophageal and gastric cancers.[27] However, we

also did not find significant difference in salivary P53 level in OSCC patients and control group.

Based on our findings and other similar studies, salivary MMP-3 and P53 levels might not be accurate enough to detect early stages of OSCC. But there are controversial statements in this regard, and we think that more researches with larger statistical samples are needed to investigate the P53 and MMP-3 expression in OSCC. ■

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med. 2012;41:736–740.
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
4. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res. 1996;56:2488–2492.
5. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res. 2000;6:357–362.
6. Sturgis EM, Wei Q. Genetic susceptibility–molecular epidemiology of head and neck cancer. Curr Opin Oncol. 2002;14:310–317.
7. Radhika T, Jeddy N, Nithya S, Muthumeenakshi RM. Salivary biomarkers in oral squamous cell carcinoma - an insight. J Oral Biol Craniofac Res. 2016;6:S51–S54.
8. Kaufman E, Lamster IB. The diagnostic applications of saliva–a review. Crit Rev Oral Biol Med. 2002;13:197–212.
9. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer. 2002;2:161–174.
10. Chen Y, Zhang W, Geng N, Tian K, Jack Windsor LJ. MMPs, TIMP-2, and TGF-β1 in the cancerization of oral lichen planus. Head Neck. 2008;30:1237–1245.
11. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res. 2003;92:827–839.
12. Liu SY, Liu YC, Huang WT, Huang GC, Su HJ, Lin MH. Requirement of MMP-3 in anchorage-independent growth of oral squamous cell carcinomas. J Oral Pathol Med. 2007;36:430–435.
13. Varun BR, Nair BJ, Sivakumar TT, Joseph AP. Matrix metalloproteinases and their role in oral diseases: a review. Oral Maxillofac Pathol J. 2012;3:186–191.
14. Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. Oral Dis. 2004;10:311–318.
15. Stokes A, Joutsa J, Ala-Aho R, Pitchers M, Pennington CJ, Martin C, et al. Expression profiles and clinical correlations of degradome components in the tumor microenvironment of head and neck squamous cell carcinoma. Clin Cancer Res. 2010;16:2022–2035.
16. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature. 1991;351:453–456.
17. Lee JJ, Kuo MY, Cheng SJ, Chiang CP, Jeng JH, Chang HH, et al. Higher expressions of p53 and proliferating cell nuclear antigen (PCNA) in atrophic oral lichen planus and patients with areca quid chewing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;99:471–478.
18. Biramijamal F, Allameh A, Mirbod P, Groene HJ, Koomagi R, Hollstein M. Unusual profile and high prevalence of p53 mutations in esophageal squamous cell carcinomas from northern Iran. Cancer Res. 2001;61:3119–3123.
19. Zhang C, Li C, Zhu M, Zhang Q, Xie Z, Niu G, et al. Meta-analysis of MMP2, MMP3, andMMP9 promoter polymorphisms and head and neck cancer risk. PLoS One. 2013;8:e62023.
20. Stott-Miller M, Houck JR, Lohavanichbutr P, Méndez E, Upton MP, Futran ND, et al. Tumor and salivary matrix metalloproteinase levels are strong diagnostic markers of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2011;20:2628–2636.
21. Agha-Hosseini F, Mirzaii-Dizgah I, Mahboobi N, Shiorazian S, Harirchi I. Serum and saliva MMP-3 in patients with OLP and Oral SCC. J Contemp Dent Pract. 2015;16:107–111.
22. Taghavi N, Biramijamal F, Sotoudeh M, Moaven O, Khademi H, Abbaszadegan MR, et al. Association of p53/p21 expression with cigarette smoking and prognosis in esophageal squamous cell carcinoma patients. World J Gastroenterol. 2010;16:4958–4967.
23. Tavassoli M, Brunel N, Maher R, Johnson NW, Soussi T. P53 antibodies in the saliva of patients with squamous cell carcinoma of the oral cavity. Int J Cancer. 1998;78:390–391.
24. Agha-Hosseini F, Mirzaii-Dizgah I, Miri-Zarandi N. Unstimulated salivary p53 in Patients with oral lichen planus and squamous cell carcinoma. Acta Med Iran. 2015;53:439–443.
25. Schoelch ML, Regezi JA, Dekker NP, Ng IO, McMillan A, Ziober BL, et al. Cell cycle proteins and the development of oral squamous cell carcinoma. Oral Oncol. 1999;35:333–342.
26. Kuropkat C, Venkatesan TK, Caldarelli DD, Panje WR, Hutchinson J, Preisler HD, et al. Abnormalities of molecular regulators of proliferation and apoptosis in carcinoma of the oral cavity and oropharynx. Auris Nasus Larynx. 2002;29:165–174.
27. Figueroa JD, Terry MB, Gammon MD, Vaughan TL, Risch HA, Zhang FF, et al. Cigarette smoking, body mass index, gastro-esophageal reflux disease, and non-steroidal anti-inflammatory drug use and risk of subtypes of esophageal and gastric cancers by P53 overexpression. Cancer Causes Control. 2009;20:361–368.