Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
PubMed (National Library of Medicine): ID: (101671502)
Research Article
Volume 8, Issue 3: May-June: 2019, 123-131
ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

EXPRESSSION OF SERUM AND SALIVARY MMP-1 AND MMP-9 IN ORAL CANCER, ORAL POTENTIALLY MALIGNANT DISORDERS AND NORMALS.

Dr. Sona Baburathinam[1], Dr. Vidyarani Shyamsundar[2], Dr. Aravindha Babu N[3], Dr. Karthika Nagarajan[4], Dr. Vijaylakshmi Ramshankar[5], Dr. Masthan K.M.K[6]

[1]Postgraduate, Department of Oral Pathology and Microbiology, Bharath Institute of Higher Education and Research, Sree Balaji Dental College and Hospitals.

[2] Professor, Department of Oral Pathology and Microbiology, Center for Oral Cancer Prevention Awareness and Research, Bharath Institute of Higher Education and Research, Sree Balaji Dental College And Hospitals.

[3] Professor, Department of Oral Pathology and Microbiology, Center for Oral Cancer Prevention Awareness and Research, Bharath Institute of Higher Education and Research, Sree Balaji Dental College And Hospitals.

[4]Reader, Department of Oral Pathology and Microbiology, Center for Oral Cancer Prevention Awareness and Research ,Bharath Institute of Higher Education and Research, Sree Balaji Dental College And Hospitals.

[5] Associate Professor, Department of Preventive Oncology, Cancer Institute (WIA), Adyar, Chennai.

[6] Professor & Head of the Department, Department of Oral Pathology and Microbiology, Center for Oral Cancer Prevention Awareness and Research, Bharath Institute of Higher Education and Research, Sree Balaji Dental College And Hospitals.

Article Info: Received 18May 2019; Accepted 15June. 2019

DOI: https://doi.org/10.32553/jbpr.v8i3.619

Address for Correspondence: Dr. Vidyarani Shyamsundar, Department of Oral Pathology and Microbiology, Center for Oral Cancer Prevention Awareness and Research, Bharath Institute of Higher Education and Research, Sree Balaji Dental College And Hospitals. Tamilnadu, Chennai- 600100

Conflict of interest statement: No conflict of interest

ABSTRACT:

Background: Matrix metalloproteinases (MMPs) have been implicated in aggressiveness, invasiveness and metastasis of many malignancies including oral squamous cell carcinoma. In the meta-analysis of microarray based Oral Tongue SCC gene expression profiles, MMP-1 and MMP-9 was highly up-regulated .Till date, non-invasive diagnostic aid to detect the malignant potential of OPML or the aggressiveness of OSCC is lacking. With this background we decided to see the level of MMP-1 and MMP- 9 in saliva and serum of OSCC patients with controls.

Aim: To determine the level of MMP1 and MMP9 in serum and saliva of patients diagnosed with OSCC, OPML, normal and to correlate with the aggressiveness of the disease.

Methodology: The epidemiologic data of the patients reporting to our dental college & Hospital were obtained through an interview. After obtaining written consent from the patient, complete intra oral examination was done, blood, saliva was collected and stored using the standard protocol. ELISA was done on all the samples for MMP-1 and MMP9.

Results: MMP-1 in serum and MMP-9 in saliva/ serum was up-regulated in OSCC, moderately up-regulated in OPML when compared to the control group. However MMP-1 in saliva was found to be very low or non detectable.

Conclusion: The MMP-9 in saliva/serum and MMP-1 in serum was up-regulated in the OSCC. In future, may be these body fluids can be used to diagnose and to determine aggressiveness of OPML and OSCC by non-invasive techniques.

Keywords: Enzyme-Linked Immunosorbent Assay (ELISA), Matrix Metalloproteinase (MMP),Oral Potentially Malignant Lesions (OPML), Oral Squamous Cell Carcinoma(OSCC), Oral Tongue Squamous Cell Carcinoma (OTSCC).

Introduction

Oral cancer is the sixth most common cancer, with an annual incidence of 36.2 million and approximately 8.2 million deaths per year all over the world. Oral Squamous cell carcinoma (OSCC)is the most common type of Oral Cancer.OSCC is a major public health concern in India due to increasing trend in incidence, increasing number of cases occurring in younger age group and its occurrence in patients without tobacco habit [1]**.**In India Among males and females, 64,225 and 33,668 respectively, new oral cases were reported in 2011. The number of cases is expected to increase exponentially to 100,389 among males and 54,458 cases among females by 2026 [2].The annual incidence rate of oral cancer is 12.6 per 100,000 population with a death of about 2000 patients/day in India [3].

The tobacco habit either chewing or smoking is the common etiologic factor and alcohol habit is the co-factor [4]. In India oral cancer is common among low social economic status which is directly linked to poor education, lack of nutrition, ignorance towards health care, poor living condition and risk behaviors. [5]. Due to lack of awareness and ignorance most of the cases are reported in advanced disease stage resulting in poor survival. Only 15% of the patients are diagnosed when the disease is at a localized stage [6]

Oral cancer patients have very high chance of developing recurrence/ second primary after the painstaking treatment. The 3-year recurrence-free survival rates were 82% for patients with no risk factors, 76% for patients with one or two risk factors and 45% for patients with three or more risk factors [7]. At least 50% of patients with locally-advanced head and neck cancer develop loco regional or distant relapses, which are usually detected within the first two years of treatment. [8]. The recurrence can be in the primary site; ipsilateral neck and contra lateral neck [9]. Neck recurrence and Second Primary Tumor were associated with 48.4% and 24.4% lower 5-year survival rates, respectively [10].

Most of the Oral cancers are preceded by clinically detectable potentially malignant disorders. The important cellular changes at the tissue level being the degree of dysplasia[11]. The potentially malignant disorder have protracted clinical course and without biopsy clinicians will not be able to judge the level of the dysplastic changes and its progression towards oral cancer. It is clinically impractical to do biopsy multiple times. Cancer cell invasion, metastasis, and angiogenesis is a multistep process involving the cooperation of multiple proteolytic enzymes secreted by tumor or host cells whose substrate include extra cellular matrix component like MMP[12]. MMPs action in the cell-to-cell adhesion and cell-to-extracellular matrix adhesion is responsible for the promotion of malignancy [13].

In our previous study on meta-analysis of OTSCC microarray based gene expression profiles, it was found MMP-1 and MMP-9 was highly upregulated and on IHC validation in tissues MMP-9 was over expressed by the cancer cells whereas the normal epithelial cells showed complete absence of MMP 9 expression [14]. The role of MMP-1 and MMP-9 is summarized in Table1. Even with the advent of medical field and enormous scientific research, no specific non invasive diagnostic aid is available to detect the malignant potential or the recurrence. The research on non-invasive technique to determine the malignant potential and recurrence is the need of time. With this background we decided to see the level of MMP-1 and MMP-9 in serum and saliva.

Materials and Methods:

The aim and objectives of the study was to determine the expression of MMP-1 and MMP-9 in serum and saliva of patients diagnosed with Oral squamous cell carcinoma (OSCC), Oral potentially malignant lesions, normal patients and to correlate with the aggressiveness of the disease.

Methodology:

The patients reporting to our college screening desk in our institution was screened to detect the presence of the specific lesions. Total of 44 patients were be included in the study. The patients were grouped into Patients with Oral squamous cell carcinoma with tobacco habits (n=20), Patients with potentially malignant disorders with tobacco habits (n=10), Patients with tobacco habit without lesion or malignancy (n=7), Age and sex matched controls without habits and without lesions (n=7).

Patient who are not willing to participate in the study, Patients diagnosed with HIV, HBsAg, and HCV infection, Patients with Oral submucous fibrosis and Terminally ill patients were excluded.

From patients included in the study, complete demographic profile, habit history was obtained. After obtaining written informed consent, salivary samples and serum samples was collected. Non-stimulatory saliva with protease inhibitor in the ratio of 1:4 was taken. These samples were centrifuged at 4000rpm for 5min. The supernatant saliva and serum was aliquoted and storage at −80°C. The severity of the disease was determined by biopsy. Specific Enzyme-linked Immunosorbent Assay (**ELISA**) on saliva and Serum samples was performed to detect the expression of MMP-1 and expression of MMP-9 Using Manufacturer Protocol. [Elabscience; Human MMP-1 ELISA KIT & Elabscience; Human MMP-9 ELISA KIT].

ASSAY PROCEDURE: The reagents like wash buffer solution, standard working solution(serial dilution method), Biotinylated detection antibody working solution, concentrated HRP conjugate working solution were prepared according to manufacturer's protocol.

Standard working solution of different concentrations was added to the first two columns of well. The saliva and serum samples were added in duplicates to the bottom of the micro ELISA plate well. The plate was covered with sealer and incubated for 90 min at 37℃. The liquid from each well was aspirated, without any wash. Immediately of Biotinylated Detection Antibody working solution was added to each well. The plate was covered with sealer and incubated for 1 hour at 37°C.The solution from each well was decanted and washing was done with wash buffer to each well. Washing was repeated 3 times. HRP Conjugate working solution was added to each well. The plate was covered with sealer and incubated for 30 min at 37℃. The solution from each well was decanted and washing was done with wash buffer to each well. This wash step was repeated 5 times. Substrate Reagent was added to each well. The plate was covered with sealer provided in the kit and incubated in dark for 15 min at 37°C. Stop Solution was added to each well. The stop solution was added in the same order as the substrate solution. The optical density (OD value) of each well at once was determined using a micro-plate reader using 450 nm filter. The acquired MEAN (Y) and OD values were entered in an excel spread sheet. The R^2 value was calculated using excel spread sheet. Linear regression equation was drawn to calculate The concentrations(x) of the MMP-1 & MMP-9 and statistical analysis was done.

All statistical analysis was performed on SPSS20.0 software. Data was expressed as Mean ± SD. Enumerate data was tested by chi-square test, whereas measurement data was presented as mean ± standard deviation and tested by ANOVA. Logistic regression model was applied for multivariate analysis. $P < 0.05$ was considered as statistically significant.

RESULTS:

The result showed, the concentration of serum MMP-1(TABLE2 & TABLE 2.1) and salivary MMP-1 (TABLE 3& TABLE3.1) was less expressed in all groups. There was no significant difference in the level of expression of MMP- 1 among different groups of patients.

The concentration of serum MMP-9(TABLE4 &TABLE 4.1) and salivary MMP-9 (TABLE 5 & TABLE5.1) was increased with significant difference in level of salivary MMP-9 when compared to the OSCC, OPML and control groups.

SERUM MMP-9 COMPARISION WITHIN GROUPS

The serum concentration of MMP-9 was compared between cancer, Oral Potentially Malignant Lesions, controls and absolute controls. Even though the serum level of MMP-9 was elevated in cancer cases, the expression was not statistically significant (Tukey Test) when compared to Oral Potentially Malignant Lesions and controls (P value-0.911, 0.91, 0.49, 0.417 respectively). The comparison of serum MMP-9(one-way ANOVA) within the study groups was also not statistically significant (0.353).[TABLE 4 & TABLE 4.1]

SALIVA MMP-9 COMPARISION WITHIN GROUPS

The saliva concentration of MMP-9 was compared between cancer, Oral Potentially Malignant Lesions, controls and absolute controls. The level of MMP-9 was elevated in cancer, Oral Potentially Malignant Lesions, control and absolute control cases. The expression between the Oral Potentially Malignant Lesions and the controls was statistically

significant with a p value of 0.046 (Tukey Test). The comparison of salivary MMP-9 (one-way ANOVA) within groups was also statistically significant (p value of 0.046).[TABLE 5 & TABLE 5.1]

Table1: Role of MMP-1 and MMP-9

MMP	ACTIVITY	EFFECT
CELL TO CELL		
MMP1	Cleavage of IGF binding protein	Proliferation
MMP9	Activation of TGF-β	Proliferation
TUMOR ANGIOGENESIS AND VASCULOGENESIS		
MMP1 AND MMP9	Activation of TGF	Activation
	Degradation of COLLAGEN-IV AND release of VEGF and Bfgf	Upregulation of angiogenesis
	Degradation of COLLAGEN-IV , COLLAGEN XVIII, Perlecan, generation of Tumstatin, Endostatin, Angiostatin And Endorepellin	Down regulation of angiogenesis
CELL ADHESIVE,MIGRATION AND EPITHELIAL MESENCHYMAL TRANSITION		
MMP1	Shedding of E cadherin	Induction of EMT; cell migration
MMP9	Over expression ,related to EMT	
IMMUNE SURVELLIANCE		
MMP1	Release of a 1 protenase inhibitor	Decrease cancer cell sensitivity to NK cells
MMP9	Shedding of interleukin-2 receptor-α by T lymphocyte surface	Supression of T lymphocyte reaction against cancer cells.

Table 2: SERUM MMP -1 COMPARISION WITHIN GROUPS

Group	Group	Mean Difference	Std. Error	Sig.
Cancer	PML	.14294	.190896	.876
	Control	-.35967	.223846	.400
	Abs. Control	-.04915	.223846	.996
PML	Control	-.50261	.223846	.149
	Abs. Control	-.19210	.223846	.826
Control	Abs. Control	.31051	.252532	.617

Table 2.1: SERUM MMP -1 COMPARISION WITHIN GROUPS (ONE-WAY ANOVA)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.652	4	.217	1.704	.202
Within Groups	2.296	18	.128		
Total	2.948	22			

Table3: SALIVA MMP -1 COMPARISION WITHIN GROUPS

Group	Group	Mean Difference	Std. Error	Sig.
Cancer	PML	.07118	.036572	.245
	Control	-.02877	.042885	.907
	Abs. Control	-.03010	.042885	.895
PML	Control	-.09995	.042885	.128
	Abs. Control	-.10127	.042885	.121
Control	Abs. Control	-.00133	.048380	1.000

Table 3.1: SALIVA MMP -1 COMPARISION WITHIN GROUPS (ONE-WAY ANOVA)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.039	4	.013	2.795	.070
Within Groups	.084	18	.005		
Total	.124	22			

Table 4: SERUM MMP -9 COMPARISION WITHIN GROUPS

Group	Group	Mean Difference	Std. Error	Sig.
Cancer	PML	-51.64286	78.464122	.911
	Control	-133.35714	92.007338	.487
	Abs. Control	-144.79464	92.007338	.417
PML	Control	-81.71429	92.007338	.811
	Abs. Control	-93.15179	92.007338	.744
Control	Abs. Control	-11.43750	103.798276	1.000

Table 4.1: SERUM MMP -9 COMPARISION WITHIN GROUPS (ONE-WAY ANOVA)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	74895.000	4	24965.000	1.159	.353
Within Groups	387866.958	18	21548.164		
Total	462761.957	22			

Table 5: SALIVA MMP -9 COMPARISION WITHIN GROUPS

Group	Group	Mean Difference	Std. Error	Sig.
Cancer	PML	-25.32143	107.370770	.995
	Control	335.22321	125.903388	.069
	Abs. Control	-4.02679	125.903388	1.000
PML	Control	360.54464(*)	125.903388	.046
	Abs. Control	21.29464	125.903388	.998
Control	Abs. Control	-339.25000	142.038178	.115

Table 5.1-SALIVA MMP -9 COMPARISION WITHIN GROUPS (ONE-WAY ANOVA)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	394195.214	4	131398.405	3.256	.046
Within Groups	726294.379	18	40349.688		
Total	1120489.594	22			

DISCUSSION:

Matrix metalloproteinase are a family of zinc dependent protease, which are secreted as proenzyme (latent enzyme) and require proteolytic cleavage for activation [15]. Digestion of the sub endothelial basement membrane is the first step toward invasion and metastasis [16]. Type IV collagen is the main component of basement membrane [17], and degradation of this structural protein is favored by metalloproteinase, namely, gelatinase-B (MMP-9) [16] and MMP 1[14]. Matrix metalloproteinase-1 (MMP-1) promotes tumor cell invasion by degrading the mesenchyma and vascular endothelium[18]Matrix metalloproteinases (MMPs) are secreted by macrophages, neutrophils and fibroblasts due to the stimulus from the transforming growth factor β (TGF-β) and interleukin-8 (IL-8). Hence, secreted MMPs maintain the bioavailability of growth factors, thus promoting cancer proliferation. It promotes and inhibits angiogenesis. [19]. MMPs action in the cell-to-cell adhesion and cell-to-extracellular matrix adhesion is responsible for the promotion of malignancy [13].

The metastatic spread of oral cancer either to local or distant vital structures is a major clinical problem and is responsible for a majority of cancer related deaths [20]. Proteolytic degradation of ECM is an essential part of this process and several enzyme systems like MMPs, serine proteinases, and cysteine proteinases have been shown to be involved[21]. MMPs play a major role in tumorigenesis, angiogenesis, and metastasis by degradation the underlying basement membrane [22]. MMP-1 and MMP-9 play an important role in its degradation because of their ability to destroy type IV collagen [23]. Several studies have shown that gelatinases are over-expressed in head and neck carcinoma cells, and play a crucial role in progression and invasion of tumors.

In this study, total number of patients included were 22(n=22). Among which 80% where male and 20% were female. The age of all the patients where above 45 years. This study shows there was no significance in the level of expression of MMP irrespectively to age and gender.

Chang et al in 2013[24] studied the association and prognostic value of serum inflammation markers in patients with oral cancer (n=151) and Leukoplakia (n=46) using ELISA and showed that the level MMPs including MMP-9 was significantly elevated (p= 0.509) and correlated with the disease progression. In current study we found a level of MMP-9 was elevated in SCC compared to Oral Potentially Malignant Lesions and normal. But the difference was statistically not significant (p=0.353).

Fathi et al 2013[25] studied CD4+CD25+T regulatory cells and MMP-9 as diagnostic salivary biomarkers in Lichen Planus(n=20) and cancer (n=10) using RT-PCR and concluded that CD4 and CD8 did not show any difference but MMP-9 levels were significantly higher in oral lichen planus and were statistically significant($p \leq 0.05$). Fathi et al stated that the main function of MMP-9 is regulation of cell matrix composition. MMP-9 cleaves denatured collagen and type IV collagen, which is the major component of the basement membrane. In our current study the levels of MMP-9 in saliva was analyzed and found to be significantly higher in SCC when compared to Oral Potentially Malignant Lesions and controls. Till date, no study has been carried out to determine the expression level of salivary MMP-9 in OSCC or in Oral Potentially Malignant Lesions with dysplasia. The study done by Fathi et al was on salivary MMP9 detected using RT-PCR and it proves that the MMP-9 was secreted in saliva.

Rajendran et al 2006[26] studied the immunohistochemical expression, gelatin zymography of MMPs in tissue samples of patients with OSF (n=20) and normal controls (n=20) and concluded that the immunoreactivity of MMP-1 shows positive stromal staining in 95% of the cases with statistical significance (p=0.006) and epithelial expression in 45% of the cases with no statistical significance (p=0.79) Of OSF. Immunoreactivity of MMP-9 showed 100% stromal staining and 20% epithelial expression. They concluded that excessive collagen deposition of the disease and the degradative enzyme levels could be the cause of excessive stromal elements. Gelatin zymography showed a decreased intensity of bands for MMP-9 (active and inactive enzyme - gelatinase) in OSF samples when compared to normal mucosa. However this could not be explained.

Paulusova et al[27], De Carvalho Fraga et al[28], Tortorici et al[29], Chen et al[30], studied the

immunohistochemical expression of MMPs in tissue samples of patients with OSCC, Oral Potentially Malignant Lesions and concluded with strong expression of MMPs in epithelium and stroma including MMP-1 & MMP-9 when compared with normal mucosa. They stated that the metalloproteinase, MMP-9, were responsible for cleaving and activating certain growth factors, including VEGF. Metalloproteinases (MMPs), also known as matrixinas due to the fact that they are Zn2+-dependent pep-tides, and are responsible for the degradation of basement membrane because they interfere with the metabolism of normal interstitial tissue.

Till date, many studies have been carried out to determine the expression level of MMP-1 and MMP-9 in OSCC, in Oral Potentially Malignant Lesions with dysplasia in a heterogeneous group of population. Also non-invasive diagnostic aid to detect the malignant potential of OPML or the aggressiveness of OSCC is lacking. Hence there was a need of homogenous studies with saliva and serum samples of same patients.

This is the first study in its nature to determine the expression level of MMP-1 and MMP-9 in serum and saliva of patients diagnosed with OSCC, Oral Potentially Malignant Lesions, normal and to correlate with the aggressiveness of the disease. The present study revealed that the serum and salivary concentration of MMP-9 was upregulated in cancer compared to OPML or controls. Saliva or serum MMP-1 had no significant difference in the level of secretion. This study signifies that the salivary MMP-9 concentration may be used as a non invasive diagnostic tool to correlate the aggressiveness of the lesion.

CONCLUSION

This study is the first to initiate NON INVASIVE DIAGNOSTIC AID to determine the expression level of secreted MMP-1 and MMP-9 in serum and saliva of patients diagnosed with OSCC, Oral Potentially Malignant Lesions, normal and to correlate with the aggressiveness of the disease. This study concludes that the expression of the MMP-9 in saliva was in par with the concentration of the serum. Hence saliva can be considered as a non invasive diagnostic tool to determine the level of secretary MMPs. Due to the technical difficulty in handling saliva and storing it without the degradation of the content, minimally invasive sample like serum/plasma was preferred to evaluate the concentration and quantify its expression in Oral Potentially Malignant Lesions and OSCC. Further studies in larger sample and with more sensitive diagnostics aids like NANO-ELISA can be preferred for more accurate determination of the aggressiveness of the disease. Hence, saliva can also be used as a non invasive diagnostic fluid for mass screenings and also as a NON INVASIVE DIAGNOSTIC AID in determining the progression/aggressiveness of Oral Potentially Malignant Lesions/ OSCC. However further studies are required in different stages of disease to correlate the aggressiveness and with the larger sample size.

Ethical clearance- Acquired (Institutional Ethical committee, Sree Balaji Dental College and Hospital). Ref NO.SBDCH / IEC/ 08/ 2017 / 1.

REFERENCES:

1. Krishnamurthy A, Ramshankar V.Early stage oral tongue cancer among non-tobacco users--an increasing trend observed ina SouthIndian patient population presenting at a single centre. Asian Pac J Cancer Prev 2013; 14(9): 5061-5.
2. Raj S, Piang LK, Nair KS, Tiwari VK, Kaur H, Singh B. Awareness regarding risk factors, symptomsand treatment facilities for cancer i n selected states of India. Asian Pac J Cancer Prev 2012-13 (8): 4057-4062.
3. Comparison of cancer incidence and patterns. http://ncdirindia.org/ncrp/PBCR_2006_2008/ Preliminary_Pages.pdf
4. Varshitha . A. Prevalence of Oral Cancer in India.J. Pharm. Sci. & Res., 2015,Vol. 7(10), 845-848.
5. Rao, Sree Vidya Krishna; Mejia, Gloria; Roberts-Thomson, Kaye; Logan, Richard "Epidemiology of Oral Cancer in Asia in the Past Decade- An Update (2000-2012)". Asian Pacific journal of cancer prevention: APJCP; 2013,14(10):5567-77.
6. Beena P. Patel, Shakuntala V. Shah, Shilin N. Shukla, Pankaj M. Shah, Prabhudas S. Patel, Clinical significance of MMP-2 and MMP-9 in patients with oral cancer, Head Neck;2007 Jun;29(6):564-72.

7. Fan KH, Wang HM, Kang CJ, Lee LY, Huang SF, Lin CY, et al.. Int J RadiatOncolBiol Phys. 2010 Jul;77(4):1024-9.

8. Weber RS, Berkey BA, ForastiereAet al. Outcome of salvage total laryngectomy following organ preservation therapy: the Radiation Therapy Oncology Group trial 91-11, Arch Otolaryngol Head Neck Surg, 2003 Jan;129(1):44-9.

9. Kernohan MD, Clark JR, Gao K, Ebrahimi A, Milross CG. Predicting the Prognosis of Oral Squamous Cell Carcinoma After First Recurrence. Arch Otolaryngol Head Neck Surg.2010 Dec;136(12):1235-9

10. Chih-HsienLiu, Han-JuChen, Pa-ChunWang, Huan-SenChen, Yen-LiangChang. Patterns of recurrence and second primary tumors in oral squamous cell carcinoma treated with surgery alone.The Kaohsiung Journal of Medical Sciences ;2013 Oct;29(10):554-9

11. Anirudh Shukla et al, Potentially malignant disorders of the oral cavity: a clinical study. Indian J Otolaryngol Head Neck Surg. 2014 Jan; 66(1): 79–85.

12. Mignatti P, Ritkin DB. Nonenzymatic interactions between proteinases and the cell surface:novel role in normal and malignant cell physiology. Adv cancer res.2007;78:103-157.

13. Venugopal A, Uma Maheswari TN. Expression of matrix metalloproteinase-9 in oral potentially malignant disorders: A systematic review.J Oral Maxillofac Pathol. 2016 Sep-Dec;20(3):474-479

14. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, et al. (2016) Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations.PLoS One. 2016 Jun 9;11(6):e0156582.

15. Birkedal HH, Moore WGI, Bodden MK, et al. Matrix metalloproteinases. A review. Crit Rev Oral Biol Med; 1993 Jan ;4(2):197-250.

16. Yoshizaki T, Maruyama Y, Sato H, Furukawa M. Expression of tissue inhibitor of matrix metalloproteinase-2 correlates with activation of matrix metalloproteinase-2 and predicts poor prognosis in tongue squamous cell carcinoma. Int J Cancer (PredOncol); 2001 Jan 20;95(1):44-50.

17. Hirota J, Yoneda K, Osaki T. Basement-membrane typeIV collagen in oral squamous cell carcinoma. Head Neck;1990 Sep-Oct; 12(5):400-5.

18. Meneses-García A, Betancourt AM, Abarca JH, Montes AB, Roa LS, Ruíz-Godoy L Expression of the metalloproteases MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, TIMP-1 and TIMP-2 in angiocentricmidfacial lymphomas .World J SurgOncol. 2008 Oct 27; 6:114.

19. Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J ;2011 oct ;278:16-27

20. Roomi MW, Monterrey JC, Kalinovsky T, Rath M, Niedzwiecki A. Patterns of MMP-2 and MMP-9 expression in human cancer cell lines. Oncol Rep. 2009;21:1323–33.

21. Hong Q, Jun T, Lei J, Xiling J, Tamamura R. Expression and clinical significance of matrix metalloproteinase-2 and its inhibitor TIMP-2 in oral squamous cell carcinoma. J Hard Tissue Biol. 2006;15:54–60.

22. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol. 2000;18:1135–49.

23. Ruokolainen H, Paakko P, Turpeenniemi-Hujanen T. Expression of matrix metalloproteinase-9 in head and neck squamous cell carcinoma: a potential marker for prognosis. Clin Cancer Res. 2004;10:3110–6.

24. Chang PY, Kuo YB, Wu TL, Liao CT, Sun YC, Yen TC. Association and prognostic value of serum inflammation markers in patients with leukoplakia and oral cavity cancer. Clin Chem Lab Med 2013;51:1291-300.

25. Fathi MS, El Dessouky HF, Breni HA. CD4+CD25+T regulatory cells and MMP-9 as diagnostic salivary biomarkers in oral lichen planus. Egypt J Immunol 2013;20:39-53.

26. Rajendran R, Rajeesh MP, Shaikh SS, Pillai MR. Expression of matrix metalloproteinases (MMP-1, MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in oral submucous fibrosis. Indian J Dent Res 2006;17:161-6.

27. Paulusová V, Laco J, Drízhal I, Slezák R. Expression of matrix metalloproteinase 9 in

patients with oral lichen planus. Acta Medica (Hradec Kralove) 2012;55:23-6.

28. de Carvalho Fraga CA, Farias LC, de Oliveira MV, Domingos PL, Pereira CS, Silva TF, et al. Increased VEGFR2 and MMP9 protein levels are associated with epithelial dysplasia grading. Pathol Res Pract 2014;210:959-64.

29. Tortorici S, Mauro A, Burruano F, Difalco P, Leone A, Gerbino A. Matrix metalloproteinase-2 matrix metalloproteinase-9 and inducible nitric oxide synthase in oral leukoplakia: Immunohistochemistry and RT-PCR analysis. J Biol Regul Homeost Agents 2008;22:125-30.

30. Chen Y, Zhang W, Geng N, Tian K, Jack Windsor L. MMPs, TIMP-2, and TGF-beta1 in the cancerization of oral lichen planus. Head Neck 2008;30:1237-45