International Journal of Research in Medical Sciences
Kherde P et al. Int J Res Med Sci. 2020 Feb;8(2):636-643
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20200248

Original Research Article

Serum levels of matrix metalloproteinase-2 in patients with oral submucous fibrosis and oral squamous cell carcinoma

Pooja Kherde*, Suchitra Gosavi

Department of Oral Pathology, Government Dental College and Hospital, Nagpur, Maharashtra, India

Received: 10 December 2019
Revised: 20 December 2019
Accepted: 03 January 2020

***Correspondence:**
Dr. Pooja Kherde,
E-mail: drpooja1204@yahoo.com

ABSTRACT

Background: Matrix metalloproteinase-2 (MMP-2), which is supposed to enable cancer cells cross the basement membrane and metastasize by selectively cleaving type IV collagen, is anticipated to be a good diagnostic and prognostic marker in oral squamous cell carcinoma (OSCC) and oral submucous fibrosis (OSMF). Thus, the study is aimed to estimate and compare the serum MMP-2 levels in patients with OSMF and OSCC.
Methods: The study was conducted on 88 subjects, divided into three groups; Group I (healthy subjects, n=28), Group II (patients with OSMF, n=30), and Group III (patients with OSCC, n=30). Serum levels of MMP-2 were estimated and compared among the groups and further with the clinical parameters within the groups.
Results: The mean serum MMP-2 levels in patients with OSMF (2.87±1.04 ng/mL) and with OSCC (11.55±2.16 ng/mL) were significantly higher than the healthy subjects (0.93±0.26 ng/mL) ($p < 0.0001$, for both). Furthermore, the mean serum MMP-2 levels in OSMF subjects had a positive association with inter-incisal opening (IIO), however, there was no association with the degree of burning sensation. Likewise, in subjects with OSCC, levels of serum MMP-2 showed positive association with histopathological grades, however, significant association with the site of occurrence and primary tumor size was not found.
Conclusions: Elevated serum MMP-2 levels can be used as a screening tool in the early detection of OSMF and OSCC cases. Moreover, MMP-2 might be a good marker in evaluating the tumor grade in OSCC and the IIO in OSMF.

Keywords: Invasion and metastasis, Matrix metalloproteinase-2, Oral squamous cell carcinoma, Oral submucous fibrosis

INTRODUCTION

Oral cancer is amongst the leading malignancies worldwide, with oral squamous cell carcinoma (OSCC) as the most common type accounting for over 90% of cases. It is the sixth most common cancer reported globally. Likewise, oral submucous fibrosis (OSMF) has been threatening a vast majority of population as an oral premalignant condition. Among the various etiopathogenic mechanisms described so far, alteration in the extra cellular matrix (ECM), brought about by numerous proteolytic enzymes and their inhibitors, is suggested to play an important role. Among the various proteolytic enzymes released by the stromal cells, matrix metalloproteinases (MMPs) play an important role in the modulation of ECM.[1] MMPs are a multi gene family of zinc-dependent endopeptidases that share a similar structure and collectively have the capacity to degrade most components of the ECM. Thus, MMPs can regulate the tumor microenvironment, and their expression and activation are increased in almost all human cancers compared with normal tissues. Based on the functional

relevance of MMPs in the pathogenesis of cancers, MMPs may be excellent biologic candidate susceptible genes for cancers. Among the MMPs, matrix metalloproteinase-2 (MMP-2), also known as gelatinase A, is a member of the MMP family that primarily hydrolyzes type IV collagen, the major structural component of basement membrane. Over expression of MMP-2 may enable cancer cells to cleave type IV collagen selectively and cross the basement membrane, allowing cancer cell entrance into the blood vessels during early stages of metastasis.[2]

Thus, this study aimed at estimation of serum MMP-2 levels in OSMF and OSCC patients and compare it with normal subjects to study the association of this marker with the pathogenesis of the lesions. This knowledge may help to improve timing of treatment and clinical efficacy.

METHODS

The study was conducted from June 2017 to November 2017 on 88 subjects who visited the outpatient department at our hospital and were divided into the following groups (Table 1).

Table 1: Categorization of all the subjects included in the study.

Groups	Subjects	Number of subjects
Group I	Healthy subjects (Control group)	28
Group II	OSMF (Clinically diagnosed)	30
Group III	OSCC (Clinically diagnosed and histopathologically confirmed)	30

Group I (the control group) included 28 apparently healthy individuals willing to participate, with no history of systemic disease, recent trauma, acute infection, burning sensation in mouth, malignancy or any treatment for previous malignancy, any other oral lesion, and any medication in the week prior to enrollment in the study. Demographic details of the subjects were recorded followed by a thorough clinical examination.

Group II (OSMF group) consisted of 30 clinically diagnosed subjects of OSMF with different amount of inter-incisal opening (IIO) and stages of progression of the disease, with no other systemic disease or prior treatment for OSMF or any premalignant lesion. Demographic details and clinical history of these subjects was recorded, along with the history of habit of consumption of areca nut and tobacco associated products.

Thorough clinical examination was performed to record blanching in oral mucosa, presence of fibrous bands in buccal mucosa, IIO, presence of any other premalignant lesion, and involvement of loco-regional lymph nodes.

The subjects were further sub-grouped as follows according to IIO, as per the Khanna and Andrarde's classification (Table 2); Group IIA: subjects with IIO >35 mm (n=2), Group IIB: subjects with IIO= 26-35 mm (n=8), Group IIC: subjects with IIO= 16-25 mm (n=16), and Group IID: subjects with IIO <15 mm(n=4).[3]

Group III (OSCC group) consisted of 30 clinically diagnosed and histopathologically confirmed cases of OSCC, with no other systemic disorder or any prior treatment for any other premalignant lesion. The locations of the tumors in the oral cavity are summarized in (Tables 3).

Table 2: Distribution of Group II subjects based on IIO.

Group	Number	Percentage
Group IIA	2	6.67%
Group IIB	8	26.67%
Group IIC	16	53.33%
Group IID	4	13.33%
Total	30	100%

Table 3: Distribution of Group III subjects based on site of OSCC.

Site	Number of subjects	Percentage
Alveolo-buccal complex	14	46.67%
Buccal mucosa	5	16.67%
Tongue	4	13.33%
Buccal Vestibule	2	6.67%
Floor of mouth	2	6.67%
Palate	2	6.67%
Commissure of lip	1	3.33%
Total	30	100%

The subjects were further sub-grouped according to primary tumor size(T), as per the TNMS classification of clinical grading; T1: subjects with T≤2 cm in greatest diameter (n=6), T2: subjects with T=2-4 cm in greatest diameter (n=18), and T3: subjects with T>4 cm in greatest diameter (n=6) (Table 4).

Table 4: Distribution of Group III subjects based on primary tumor size.

Size	Number of subjects	Percentage
T1	6	20%
T2	18	60%
T3	6	20%

Furthermore, histopathological grading of OSCC was done as per Broder's grading, as well-differentiated (WDSCC) (n=23), moderately differentiated (MDSCC) (n=6), and poorly differentiated (PDSCC) (n=1) squamous cell carcinomas (Table 5).

Table 5: Distribution of Group III subjects based on histopathological differentiation.

Histopathological grade	No. of Subjects	Percent
WDSCC	23	76.67%
MDSCC	6	20%
PDSCC	1	3.33%

Individuals with immunocompromised status or with debilitating systemic diseases, e. g., diabetes mellitus, cardiovascular diseases, hepato-biliary disorders, rheumatoid arthritis, etc., were excluded from the study.

The subjects in all the three groups were subjected to detailed clinical examination. Demographic details, dental and medical history, and history of relevant habits like consumption of betel nut, tobacco and related products was recorded.

Sample collection and MMP-2 estimation was done by collecting 3-ml of intravenous blood sample from each subject and was centrifuged in a Remi bench-top centrifuge for 15 minutes at 3,000 rpm within one hour of collection. Serum was separated (the top transparent layer) and stored in Eppendorf tubes at -20ºC in deep freezer. It was then used for quantitative estimation of serum MMP-2 by Fine Test Human MMP-2 enzyme linked immunosorbent assay (ELISA) kit and Robonik Readwell Touch ELISA plate analyzer.

Statistical analysis

Data was collected, tabulated, and analyzed by using SPSS 20© (Statistical package for Social Sciences) software. One-way ANOVA test was applied and a p value<0.05 was considered as statistically significant. Turkey's post hoc test was applied for further inter group comparisons. The results were expressed as mean, standard deviations (SD), and percentages (%).

RESULTS

Among the 88 subjects included in this study, 57 were males and 31 females. Moreover, group I constituted 10 males and 18 females, group II constituted 24 males and 6 females, and group III constituted 23 males and 07 females (Table 6).

The age range of the study population was 18-79 years (39.27±14.32 years). Moreover, the age of the subjects in group I was 21-64 years (34.46±13.09 years); in group II was 18-60 years (32.63±10.79 years), with peak occurrence of OSMF in 3rd and 4th decades of life; and in group III was 34-79 years (50.4±11.99 years, with peak occurrence of OSCC in 5th and 6th decades of life (Table 7).

The levels of serum MMP-2 in group I ranged from 0.52-1.42 ng/mL (0.93±0.26 ng/mL); in group II ranged from 1.22-4.75 ng/mL (2.87±1.04 ng/mL), and in group III ranged from 8.62-16.35 ng/mL (11.55±2.16 ng/mL) (Table 8).

Table 6: Gender-wise distribution of subjects among all groups.

		Groups		
		Group I n (%)	Group II n (%)	Group III n (%)
Sex	Male	10 35.70%	24 80%	23 76.67%
	Female	18 64.29%	6 20%	7 23.33%
Total		28 100%	30 100%	30 100%

Table 7: Age-wise distribution of subjects among all groups.

Group	Number	Mean age	Std. Deviation
Group I	28	34.46	13.09
Group II	30	32.63	10.79
Group III	30	50.4	11.99
Total	88	39.27	14.32

Table 8: Comparison of mean serum MMP-2 levels in Group I, Group II, and Group III subjects.

Group	Mean MMP-2	Std. Deviation	p value
Group I	0.93	0.26	<0.0001
Group II	2.87	1.04	
Group III	11.55	2.16	

Table 9: Comparison of mean serum MMP-2 levels in Group II subjects based on IIO.

Group	Number	Mean MMP-2	SD	p value
Group IIA	2	1.47	0.35	0.0106
Group IIB	8	2.42	1.21	
Group IIC	16	3	0.81	
Group IID	4	3.98	0.18	

The mean serum MMP-2 levels were higher in subjects with OSMF as compared to normal subjects and the difference was highly statistically significant (p <0.0001). Moreover, in the present study, maximum subjects with OSMF belonged to Group IIC, i.e., they had an IIO = 16-25 mm. The mean serum MMP-2 levels in each of the subgroups was calculated and compared, and it was found that the mean serum MMP-2 level in Group IIA was 1.47±0.35 ng/mL, in Group IIB was 2.42±1.21 ng/mL, in Group IIC was 3±0.31 ng/mL, and in Group IID was 3.98±0.18 ng/mL. One-way ANOVA test was applied, and a statistically significant difference was

found in the mean serum MMP-2 levels among the subgroups formed based on IIO (p=0.0106) (Table 9).

Furthermore, presence of burning sensation in Group II subjects was recorded and graded as absent, mild, moderate, and severe burning sensation, and it was found that out of the 30 subjects, 7(23.33%) subjects did not present any symptom of burning sensation, 14(46.67%) had mild burning sensation, 7(23.33%) had moderate burning sensation, and 2(6.67%) exhibited symptoms of severe burning sensation (Table 10).

Table 10: Distribution of Group II subjects based on burning sensation.

Burning sensation	Number	Percentage
Absent	7	23.33%
Mild	14	46.67%
Moderate	7	23.33%
Severe	2	6.67%
Total	30	100%

When the mean serum MMP-2 levels were compared among the subjects in group II with different degrees of burning sensation; it was found that in subjects with no burning sensation, the mean level was 2.64±1.15 ng/mL; in those with mild burning sensation, it was 2.79±1.02 ng/mL; in those with moderate burning sensation, it was 3.32±1.14 ng/mL; and in those with severe burning sensation, it was 2.71±0.04 ng/mL (Table 11). However, on one-way ANOVA test analysis, the difference was not found to be statistically significant (p<0.6).

Table 11: Comparison of mean serum MMP-2 levels in Group II subjects based on burning sensation.

Group	Number	Mean MMP-2	SD	p value
Absent	7	2.64	1.15	0.6370
Mild	14	2.79	1.02	
Moderate	7	3.32	1.14	
Severe	2	2.71	0.04	

The mean serum MMP-2 level in group III was 11.55±2.16 ng/mL and was higher as compared to the normal subjects, the difference being highly statistically significant (p <0.0001). Group III subjects were categorized based on site of lesion in oral cavity, as shown in (Table 3). Among the 30 subjects, 14(46.67%) subjects showed involvement of the alveolo-buccal complex, 5(16.67%) showed that of the buccal mucosa, 4(13.33%) showed that of the tongue, 2(6.67%) showed that of the buccal vestibule, 2(6.67%) showed that of the floor of the mouth, 2(6.67%) showed that of the palate, and 1(3.33%) showed that of the commissure of lip.

Mean serum MMP-2 level in subjects with alveolo-buccal complex carcinoma was 11.94±2.58 ng/mL, in those with carcinoma involving the buccal mucosa was 11.40±1.66 ng/mL, in those with that involving tongue was 11.46±2.19 ng/mL, in those with that involving buccal vestibule was 12.88±0.62 ng/mL, in those with carcinoma involving floor of mouth was 9.38±0.02 ng/mL, in those with carcinoma of palate was 11.50±0.11 ng/mL, and in those with carcinoma of commissure of lip was 8.84 ng/mL (Table 12). However, the differences were statistically insignificant according to the one-way ANOVA test (p<0.61).

Table 12: Comparison of mean serum MMP-2 levels in Group III subjects based on site.

Site	Number	Mean MMP 2	SD	p value
Alveolo-buccal complex	14	11.94	2.58	0.601
Buccal mucosa	5	11.40	1.66	
Tongue	4	11.46	2.19	
Buccal Vestibule	2	12.88	0.62	
Floor of mouth	2	9.38	0.02	
Palate	2	11.50	0.11	
Commissure of lip	1	8.84		

Furthermore, among the subgroups formed based on tumor size, the mean MMP-2 level in subjects with tumor size T1 was 11.25±2.02 ng/mL, in subjects with tumor size T2 was 11.99±1.88 ng/mL, and in subjects with tumor size T3 was 11.50±2.86 ng/mL. The difference was, however, statistically insignificant (p=0.24) (Table 13).

Table 13: Comparison of mean serum MMP-2 levels in Group III subjects based on primary tumor size.

Size	Mean MMP-2	Std. Deviation	p value
T1	10.25	2.02	0.236
T2	11.99	1.88	
T3	11.50	2.86	

Table 14: Comparison of mean serum MMP-2 levels in Group III subjects based on histopathological differentiation.

Histopathological Grade	Mean MMP-2	Std. Deviation	p value
WDSCC	10.77	1.69	0.0002
MDSCC	13.71	1.24	
PDSCC	16.35		

Additionally, the mean serum MMP-2 levels were compared based on histopathological grades of OSCC, and it was found that the mean serum MMP-2 level in subjects with WDSCC was 10.77±1.69 ng/mL, in those with MDSCC was 13.71±1.24 ng/mL, and in the one with PDSCC was 16.35 ng/mL (Table 14); the differences were highly statistically significant (p=0.0002).

Finally, serum MMP-2 levels was compared among all the three groups and a statistically significant difference was found (p<0.001) (Table 8). Moreover, on turkey's post hoc test application for further inter group comparisons, the mean serum MMP-2 level was significantly higher in subjects with OSCC (Group III) as compared to both remaining groups, i.e., the control group (Group I) and the group of subjects with OSMF (Group II) (Table 15).

Table 15: Paired comparison of serum MMP-2 levels between the groups.

Groups	Absolute mean difference	p value
Group I-Group II	2.447	<0.0001
Group I-Group III	11.118	<0.0001
Group II-Group III	8.671	<0.0001

On summarizing all the above observations, it can be stated that serum MMP-2 levels in subjects with OSMF were significantly higher as compared with normal subjects, with a positive association with IIO and no association with the degree of burning sensation.

Likewise, in subjects with OSCC, levels of serum MMP-2 were significantly higher as compared to normal individuals, and these levels showed positive association with histopathological grades. However, no significant association was observed with the site of occurrence and primary tumor size.

DISCUSSION

OSCC arises because of multiple molecular events that develop from the combined effects of an individual's genetic predisposition and exposure to environmental carcinogens such as tobacco, alcohol, chemical carcinogens, ultraviolet or ionizing radiation, and microorganisms. Chronic exposure to carcinogens may damage individual genes as well as larger portions of the genetic material, such as chromosomes. Genetic damages may activate mutations or amplification of oncogenes that promote cell survival and proliferation.[4]

In India there is an easy access to tobacco, areca nut, and associated products; and population involved with consumption of such products is considerably high. India shares a significantly high burden of oral premalignant disorders including OSMF and OSCC. In India alone, over 100,000 new cases of OSCC are registered every year. In a survey done over a decade ago, there were more than 250,000 OSMF cases recorded, a figure that must have increased sharply till date.[5]

OSMF is a premalignant condition of the oral cavity characterized by inflammation and progressive mucosal fibrosis. It affects the mucosa of any part of the oral cavity, occasionally extending into the pharynx and esophagus. The pathogenesis of the disease is not well established. Since its first description, various etio-pathological concepts have been put forward, such as spices (chilies), nutritional deficiencies, genetic susceptibility, lysyl oxidase, autoimmunity, and one of the most accepted etio-pathology, i.e., the areca nut usage.[6]

Epithelial atrophy may predispose to cancer development in the presence of carcinogens. The precancerous nature of OSMF was first described by Paymaster in 1956 when he observed slow growing squamous cell carcinoma in one third of the patients with the disease.[7]

Malignant transformation rate of OSMF is found to be different in various studies. In a long-term follow-up study by Murti et al, it was found to be in the range of 7-13%. According to another long-term follow-up study, a transformation rate of 7.6% over a period of 17 years has been reported.[6]

Processes such as cell proliferation, angiogenesis, apoptosis, or invasion are strongly influenced by the surrounding microenvironment of the tumor. Therefore, the ability to change these surroundings represents an important property through which tumor cells acquire specific functions necessary for tumor growth and dissemination. MMPs constitute key players in this process, allowing tumor cells to modify the ECM and release cytokines, growth factors, and other cell-surface molecules, ultimately facilitating protease-dependent tumor progression.[8]

MMPs are produced by a variety of cells during both physiologic conditions like normal development and wound healing and a wide variety of pathological processes. Currently, 28 human MMPs have been identified, and these enzymes have been classified according to their substrate specificity and structural similarities. The major subgroups are interstitial collagenases, gelatinases, stromelysins, and membrane-bound MMPs. They are produced by several cell types including fibroblasts, macrophages, neutrophils, and some epithelial cells. Their secretion is induced by certain stimuli including growth factors, cytokines, and physical stress. Most MMPs are synthesized as pro-peptides that require proteolytic cleavage for activation.[9]

MMP-2, a gelatinase, is most consistently linked to various carcinomas. The expression and prognostic significance of MMP-2 is not fully clarified, but recent studies implicate that increased MMP-2 expression in the primary tumor is associated with aggressive disease and unfavorable outcome. MMP-2 has been detected in serum of breast cancer, prostate cancer, colorectal cancer, and laryngeal cancer patients.[10-13] Serum MMP-2 levels have been found to be increased in them as compared to the control group.

The mean serum levels of MMP-2 in the OSMF as well as OSCC groups were higher than that of the control group, with the difference being statistically significant.

Moreover, authors compared the serum MMP-2 levels in the OSMF patients with the degree of blanching, IIO, and burning sensation. IIO in a way reflects the establishment of the disease in OSMF. OSMF starts because of chronic irritation caused by various chemicals found in areca nut, implying presence of inflammatory response, which would be acute initially and then would become chronic over a period as the disease is established.

Thus, an increase was anticipated in serum MMP-2 levels with decreased IIO, and the results supported the anticipation, with the differences in the levels being statistically significant among the subgroups with different IIOs ($p = 0.01$).

Burning sensation in OSMF relates with the atrophy of oral epithelium and degranulation of mast cells. Burning sensation is also associated with vesicle formation and release of various chemokines by mast cells.[14] MMPs modulate inflammation by regulating bioavailability and activity of cytokines, chemokines, and growth factors, as well as integrity of physical tissue barriers.[15] Thus, some changes were anticipated in serum MMP-2 levels of the subjects with OSMF based on burning sensation. Although, as anticipated, study showed high serum MMP-2 levels in Group II subjects with moderate burning sensation, the levels did not significantly increase with the degree of burning sensation ($p<0.64$). Thus, no correlation between the serum MMP-2 levels and degree of burning sensation was concluded.

To the best of knowledge, this is the first study yielding results based on these comparisons (comparisons with the clinical parameters) as well; however, for more reliable conclusions, further studies are advocated.

The mean serum MMP-2 levels were significantly higher in OSCC patients as compared to healthy individuals. This was in accordance with the findings of Patel B et al, wherein they found elevated plasma MMP-2 levels in OSCC cases when compared with the levels in normal subjects.[16]

In another recent study done by Lofti A et al, where they evaluated the serum levels of MMP-2 and MMP-9 in 20 patients with OSCC and compared them to those of 20 normal subjects, both markers were found to be significantly increased in OSCC patients compared to healthy subjects.[13]

The distribution of sites of OSCC among the subjects was similar to that proposed by Warnakulsuriya S., who also observed similar kind of occurrence of OSCC in their study, buccal cancer being more common among Asian populations due to betel quid/tobacco chewing habits.[5] When comparison was made based on the site of occurrence in subjects with OSCC, no significant difference in the mean levels of serum MMP-2 was observed. This observation can be justified because though the site of occurrence of OSCC in these subgroups may be different, the basic pathogenesis in OSCC remains the same, and hence, serum MMP 2 levels would be the same.

When comparison of mean serum MMP-2 levels was done based on tumor size, mean serum MMP-2 level was found to be highest in T2 subjects followed by T1 and T3 subjects. However, there was no statistically significant difference ($p=0.236$). Therefore, relationship between tumor size and serum MMP-2levels could not be established.

When comparison of serum MMP-2 levels in OSCC subjects was done based on histopathological grading, the levels increased from WDSCC to PDSCC with statistically significant difference ($p=0.0002$).

Similar results were obtained by Lofti A et al, in their study where the higher serum levels of MMP-2 were significantly correlated with increase in tumor grade ($p=0.001$).[13]

Lastly, when mean serum MMP-2 levels in all three groups were compared, it was seen that mean serum level was higher in subjects with OSMF than normal subjects with statistically significant difference. Likewise, a statistically significant difference in mean serum MMP-2 level was seen in OSCC subjects when compared to OSMF and normal subjects.

In the present study, found overall low levels of serum MMP-2 when compared with the study of Lofti A et al. This could be because the assay detection range used for MMP-2 was 10-3,000 ng/mL in their study; whereas, in our study we used low-concentration assay detection range, i.e., 0.5-32 ng/mL. This justifies the difference in the serum MMP-2 levels in our study when compared with the previous study.

In the present study, mean age of the subjects with OSMF (Group II) was 32.63 years, with peak occurrence observed in 3rd and 4th decades of life (Table 7). This was similar to the findings of J G Ray et al, who also found similar occurrence.[17] Likewise, the gender predominance was similar to the findings of J G Ray et al, and Shrestha A et al, who found occurrence of OSMF more in males as compared to females.[17,18]

Moreover, the mean age of the subjects with OSCC (Group III) was 50.04 years, with a peak incidence in 5th and 6th decades of life. This finding was similar to the findings of Ku K T et al, who stated the mean age of OSCC patients as 52 years.[19]

Warnakulsuriya S. commented that the risk of developing cancer increases with age, and majority of cases occur in people aged 50 years or above. However, in high incidence countries like India, a shift of this paradigm is noted towards early decades of life.[5] The gender predominance in our study was similar to that stated by

Warnakulsuriya S., who showed a marked male predominance. In most countries around the world, cancer is more common in males than females.[5] This gender difference can be explained by heavier indulgence in risk habits by men and exposure to sun as a part of outdoor occupations, over the lower proportion of risk habits in women.

The limitations of the study included small sample size and a single-center study design.

To the best of knowledge, studies in this context usually have measured immunohistochemical expression of MMPs and not their serum levels. Moreover, there seems to be no data available in literature showing a statistically significant correlation between serum MMP-2 levels and OSMF and a co-relationship between its various clinical parameters.

Increased serum MMP-2 levels was found in OSCC subjects. These increased MMP-2 levels may be attributed to degradation of ECM, leading to the invasive and metastatic potential of OSCC. This ability of MMP-2 and MMP-9 to initiate basement membrane destruction and further degrade the collagenous and non-collagenous components of the ECM suggests that they are important in processes of invasion and metastasis.

Metastatic tumor expresses more type IV collagenase activity than their non- metastatic counterparts. Therefore, production of large amount of MMP enzymes is an important factor for predicting the prognosis of patients with potentially malignant lesions like OSMF and malignancies like OSCC. Action of MMP-2 correlates well with the malignant potential of a tumor. Serum post-operative MMP 2 levels have also been reported to be predictor of disease-free survival and overall survival.[10]

Evidence also supports the view that extracellular proteinases such as the MMPs mediate many of the changes in the microenvironment during tumor progression. These enzymes are the key players in the molecular communication between tumor and stroma.[20]

A gradual increase in MMP 2 levels from well to moderate to poorly differentiated OSCC is observed in our study which is suggestive of MMP 2 has a role in the aggressiveness of tumor with increasing grades.

CONCLUSION

Elevated serum MMP-2 levels can be used as screening tool in the early detection of OSMF and OSCC cases. Moreover, MMP-2 might be a good marker in evaluating the tumor grade in OSCC and evaluating the IIO in OSMF. Targeted therapy in the form of MMP-2 inhibitors can be used against MMP-2 in future, which is currently under clinical trials. Since, however, the supporting literature is scarce to date, further studies are advocated.

ACKNOWLEDGEMENTS

Authors would like to thank the ancillary staff of the department of oral pathology.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kherde P, Gosavi S. Serum levels of matrix metalloproteinase-2 in patients with oral submucous fibrosis and oral squamous cell carcinoma. Int J Res Med Sci 2020;8:636-43.