Review Article

Expression of matrix metalloproteinase-9 in oral potentially malignant disorders: A systematic review

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Abstract

Matrix metalloproteinase-9 (MMP-9) is an inducible enzyme. Oral potentially malignant disorders (OPMDs) are considered as the early tissue changes that happen due to various habits such as smoking tobacco, chewing tobacco or stress. This alteration in the tissues alters the expression of MMP-9. The rationale of the review is to know the expression of MMP-9 in OPMDs. Hand searching and electronic databases such as PubMed and ScienceDirect were done for mesh terms such as OPMDs and MMP-9. Eight articles were obtained, after applying inclusion and exclusion criteria. These articles were assessed with QUADAS and data were extracted and evaluated. The included eight studies were done in 182 oral squamous cell carcinoma cases, 430 OPMDs (146 oral lichen planus, 264 leukoplakia and 20 oral submucous fibrosis) and 352 healthy controls evaluated for MMP-9. MMP-9 expression was found to be elevated in tissue, serum and saliva samples of OPMDs than in healthy controls. There is only one study in each serum and saliva samples to evaluate MMP-9. Saliva being noninvasive and serum being minimally invasive, more studies need to be done in both serum and saliva to establish MMP-9 as an early diagnostic marker in OPMDs to know its potential in malignant transformation.

Key Words: Leukoplakia, matrix metalloproteinase-9, oral lichen planus, oral potentially malignant disorders, oral submucous fibrosis

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INTRODUCTION

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 cleaves the FAS receptors and suppresses natural killer cells, resisting the apoptosis. It promotes and inhibits angiogenesis. In addition, it increases the bioavailability of vascular endothelial growth factor receptor (VEGFR) to cause neovascularization. Substances such as tumstatin, endostatin, angiostatin and endorepellin inhibit angiogenesis.[1] MMPs action in the cell-to-cell adhesion and cell-to-extracellular matrix adhesion is responsible for the promotion of malignancy.

The expression of MMP-9 has proved to be a diagnostic marker in oral cancer in the tissue,[2] serum[3] and saliva[4] samples in various studies. An overall incidence of cancer in Central Asia is at the rate of 100.8/100,000 in world. Oral cavity cancer claims 145,400 deaths in a year worldwide. The predisposing

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factors for oral cavity cancer include smoking and smokeless tobacco, alcohol and human papillomavirus infections.[5] Oral carcinoma develops from oral potentially malignant disorders (OPMDs).[6] Potentially malignant disorder, the term was proposed as all the conditions called to be so, does not transform into malignancy.[7] The prevalence rate of oral lesions is 4.1% in South India.[8] The rate of oral potential malignant disorder transforming to malignancy is 2%–3%.[9] OPMDs are considered as the early tissue changes due to various habits such as smoking and chewing tobacco.

There are several studies that have been done in the relationship of MMPs to cancer invasion, progression, apoptosis, migration and neovascularization in cancer. MMP-9 is an inducible enzyme, unlike MMP-1 and MMP-2 which are constitutive enzymes.[10] Hence, its levels would be altered during the changes in the tissues. The rationale of the review is to know the expression of MMP-9 in OPMD. To know which of these that is tissue, saliva or serum is the most reliable means of detecting the expression of MMP-9.

METHODOLOGY

Various databases such as PubMed and ScienceDirect were searched using the key words of OPMD, oral leukoplakia, oral submucous fibrosis (OSMF), oral lichen planus (OLP), oral cancer, MMP-9, gelatinase B, 92K Da gelatinase, 92 K Da Type IV collagenase. A total of 35 articles were identified, in which 5 article was obtained by hand searching.

Inclusion criteria

Articles in English language which reported checking of the MMP-9 levels in tissue, saliva or serum samples of OPMD during 2005–2015 were included in the study.

Exclusion criteria

Animal studies were excluded. The studies done only in oral cancer excluding the OPMDs were excluded. The studies done in cancers other than oral cancer were excluded from the study.

Applying these inclusion and the exclusion criteria, 32 articles were excluded as one of them was a study on animals, one of them was in Russian language, one article was not done with in the time period of the included study and the rest of the twenty nine were either done in a different MMP or in a different cancer or only in oral cancer and not in OPMDs [Figure 1]. A total of eight studies were obtained which were assessed by QUADAS,[11] quality assessment tool for risk of bias and acceptability concern. Data were collected using a table having all the characteristics of the included study [Table 1].

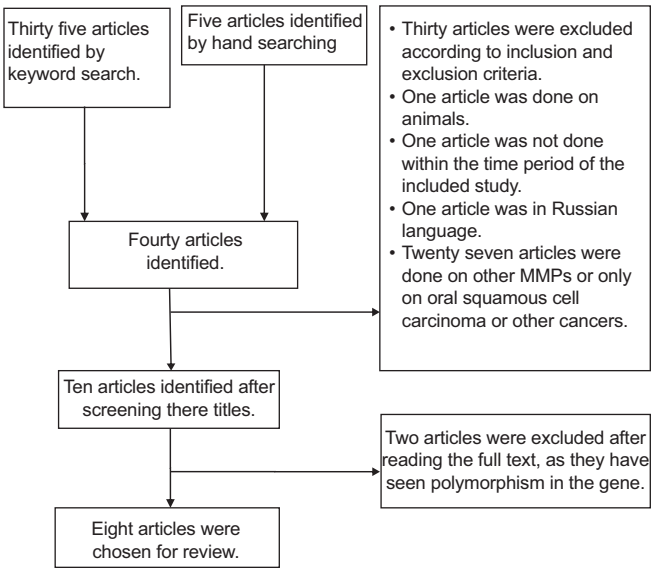

Figure 1: Prisma flowchart for selection of studies

RESULTS

These studies were done on 182 oral squamous cell carcinoma (OSCC) cases, 430 OPMDs (146 OLP, 264 leukoplakia and 20 OSMF) and 352 controls evaluated for MMP9 [Figure 2]. In all the studies except one study,[12] MMP-9 expression has been statistically proved to be elevated in OPMDs than in healthy controls. Moreover, MMP-9 expression in OPMDs was decreased than in OSCC. The study in the saliva states MMP-9 to have a sensitivity of 35.3% and specificity of 100%. The study in serum states sensitivity of 67.4% and specificity of 90%. Whereas, the studies in tissues have not given any sensitivity or specificity of MMP-9 or the cutoff value to clearly identify the condition.

DISCUSSION

MMPs occur in human samples, which can be evaluated by immunohistochemistry, enzyme-linked immunosorbent assays (ELISA), zymography and real-time polymerizing chain reaction. Immunohistochemistry is the most commonly used methodology in tissue samples,[12,15-19] which has not quantified the MMP-9 levels, it also cannot differentiate between the latent and active forms of MMP. In serum sample, only a single study has been done in which MMP-9 is quantified by ELISA,[14] which is sensitive but expensive. Gelatin zymography is cost-effective, can be reproduced and can differentiate between latent and active form of the enzyme. In the saliva samples, the method used is real-time reverse transcriptase polymerizing chain reaction to detect the genetic expression of mRNA of MMP-9,[13] which makes the technique more sensitive.

Table 1: Characteristics of included studies

Author and year	Journal	Samples		Method	Parameters measured	Statistical test	Results	
Chang *et al*., 2013[14]	Clinical Chemistry Lab Medicine	Serum* Tissue Saliva	151 - OSCC 46 - oral leukoplakia	ELISA	IL-6, M-CSF, TGF-β, ICAM-1, E-selectin, CRP, SAA, MMP-2, MMP-9	Mann–Whitney test and Spearman correlation	Oral leukoplakia TGF-β, E-selectin, CRP, MMP-2 and MMP-9 significantly elevated	OSCC IL-6, TGF-β, ICAM-1, E-selectin, CRP, MMP-2, MMP-9 are significantly elevated MMP-9 (ρ=0.509, *P*<0.001) correlation with disease progression
de Carvalho Fraga *et al*., 2014[17]	Pathology research and practice	Serum Tissue* Saliva	48 - oral leukoplakia 20 - OSCC 21 - HC	Immunohistochemistry	VEGFR2 and MMP-9	Mann- Whitney and Kruskal–Wallis test. Spearman correlation	OL Correlation was found in the in oral leukoplakia samples (*r*=+0.452, *P*=0.001)	OSCC VEGFR2-80%, MMP-9-75% expression found No correlation found (*r*=−0.042, *P*=0.861)
Paulusová *et al*., 2012[12]	Acta medica	Serum Tissue* Saliva	71 - OLP 10 - HC	Immunohistochemistry	MMP-9	Not mentioned	Mucosal fibroma - Expression was seen in the fibroblasts in endothelium of small vessels with occasional positivity in overlying epithelium	OLP - Expression was seen in the lymphocytic inflammatory infiltrate in the lamina propria and in epithelium. It was seen in the stratum basale and stratum spinosum
Fathi *et al*., 2013[13]	Egyptian journal of immunology	Serum Tissue Saliva*	20 - OLP 10 - HC	RT-PCR	CD4, CD8 and MMP-9	Chi-square test	CD4 and CD8 did not show any difference in both the groups but MMP-9 levels were significantly higher in OLP when compared to HC (*P*≤0.05)	
Chen *et al*., 2008[19]	Journal of science and specialties of head and neck	Serum Tissue* Saliva	27 - OLP 15 - OSCC 11 - HC	Immunohistochemistry and semiquantitative analysis	MMP-2, MMP-9, MT1-MMP, MMP-14, TIMP-2 and TGF-β1	Mann-Whitney test, Spearman correlation and Kruskal–Wallis	In the nonatrophic LP, MMP-2, MMP-9 and MT1-MMP in the epithelium vary from negative to moderate	In the atrophic LP MMP-2, MMP-9 and MT1-MP in the epithelium from moderate to strong	OSCC – MMP-2, MMP-9 and MT1-MP in the epithelium stained strongly
Rajendran *et al*., 2006[16]	Indian journal of dental research	Serum Tissue* Saliva	20 - OSMF 20 - HC	Immunohistochemistry and gelatin zymography	MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2	Chi-square test	MMP-9 shows positive stromal staining in 100% of OSMF cases (*P*=0.00). Zymography showed decreased intensity of bands for MMP-2 (active and inactive and MMP-9 in OSMF was compared to normal mucosa	
Tortorici *et al*., 2008[18]	Journal of biological regulators and homeostatic agents	Serum Tissue* Saliva	170 oral leukoplakia 170 healthy oral mucosa	Immunohistochemistry and RT-PCR	MMP-2, MMP-9, iNOS	Not mentioned	The distribution of MMP-9 has the same features both in healthy oral mucosa and in the leukoplakia sample; immune expression is stronger in leukoplakic tissues. In RT-PCR, the strong expression of both MMP-2 and MMP-9 in both samples	

Contd...

Table 1: Contd...

Author and year	Journal	Samples		Method	Parameters measured	Statistical test	Results
Al-Rawi *et al*., 2014[15]	Journal of orofacial sciences	Serum Tissue* Saliva	28 - OLP 6 - OSCC 6 - HC	Immunohistochemistry	MMP-2, MMP-9, TIMP-1, TIMP-2	One-way ANOVA	MMP-2, MMP-9, TIMP-1 and TIMP-2 all showed a significant rise in OLP and OSCC when compared to negative control (P=0.00, P=0.00, P=0.00 and P=0.001)

*Sample with significant results OSCC: Oral squamous cell carcinoma, OLP: Oral lichen planus, HC: Healthy control, OSMF: Oral submucous fibrosis, MMP-9: Matrix metalloproteinase-9, MMP-2: Matrix metalloproteinase-2, MMP-1: Matrix metalloproteinase-1, TIMP-1: Tissue inhibitor of matrix metalloproteinase-1, TIMP-2: Tissue inhibitor of matrix metalloproteinase-2, RT-PCR: Reverse transcription polymerase chain reaction, iNOS: Inducible nitric oxide synthase, M-CRP: Monomeric C-reactive protein, TGF-β1: Transforming growth factor beta 1, ICAM-1: Intercellular adhesion molecule-1, VEGFR2: Vascular endothelial growth factor receptor 2, SAA: Serum amyloid A, MT1: Membrane type 1

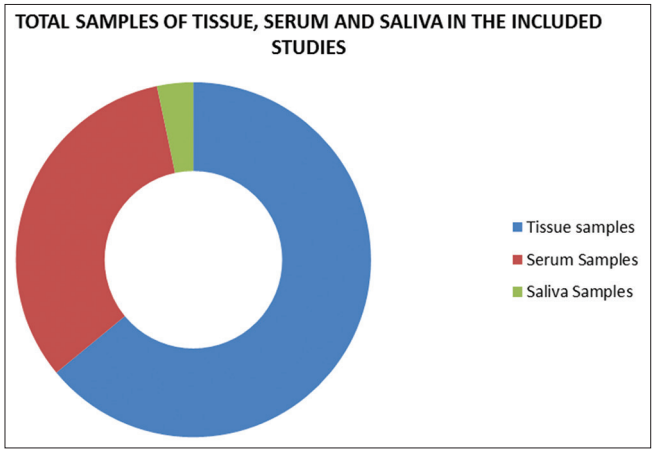

Figure 2: Total distribution chart of samples of tissue, serum and saliva in the included studies

The studies considered in this review have proved that MMP-9 has a positive correlation with VEGFR2 (r = +0.452) and epithelial dysplasia grading in oral leukoplakia samples.[17] Inflammatory markers (IL-6, M-CSF, TGF-β1, intercellular adhesion molecule-1, E-selectin, C-reactive protein [CRP], serum amyloid A, MMP-2), were analyzed in oral leukoplakia cases, showed rise in TGF-β, E selectin, CRP, MMP-2 and MMP-9 levels and the markers such as MMP-9, CRP and TGF-β correlated with disease progression. This study proved MMP-9 to have highest diagnostic power among the four markers (MMP-2, MMP-9, TGF-β and CRP) to distinguish oral leukoplakia and OSCC from healthy control.[14] In a study on OLP, a positive correlation was found between MMP-2 and MMP-9 and the expression of TGF-β showed increase with the level of MMP-9.[19] $CD4^+$, $CD25^+$ and MMP-9 levels were significantly increased in OLP when compared to healthy control group.[13] In the tissue samples of OLP, MMP-9 showed to stain the stratum basale and the stratum spinosum of the keratinocytes and this study did not test the hypothesis of the study; hence, there is a high risk of bias.[12] The stromal staining of MMP-9 in tissue samples of OSMF was 100% when compared to 20% in healthy controls. This study also states the stromal staining of MMP-2, MMP-1, tissue inhibitors of metalloproteinase-1 (TIMP-1) and TIMP-2, which were also elevated like MMP-9.[16] MMP-2, MMP-9, TIMP-1 and TIMP-2 showed a significant variation from the normal control in tissue samples of OLP.[15] The elevated level of MMP-9 posttreatment was also revealed to be a marker for recurrence of OSCC.[14]

Tissue inhibitors or TIMPs inhibit the action of MMPs. The imbalance between the MMPs and the TIMPs is one of the reasons for progression of malignancy. Of the eight studies, three studies[15,16,18] done on tissue samples have compared MMP-9 with TIMPs. Two studies have seen them in OLP and one has done in OSMF. One study on submucous fibrosis states that TIMP-1 does not give a statistically significant result while TIMP-2 does. One of the studies does not mention about the relationship of MMP-9 to TIMPs.[16] While the other study says that TIMP-1 and TIMP-2 are expressed more strongly than in the OSCC, no relation was found with the level of MMP-9.[15] MMP-9 is inhibited by all the four TIMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4).[20] Only TIMP-1 and TIMP-2 have been estimated in three of the above studies and the remaining two TIMPs (TIMP-3 and TIMP-4) have not been evaluated. There are synthetic TIMPs and also TIMPs specific to MMPs being developed,[20] which can be used in intervention of malignancies.

One study done in saliva samples (AUC-0.647)[13] [Table 2] and another done in serum samples (AUC-0.806)[14] [Table 3] have mentioned the sensitivity, specificity [Figure 3] and receiver operating characteristic (ROC) for MMP-9 [Figure 4] and other markers in them, the remaining six studies[12,15-19] [Table 4] being diagnostic tests, have not mentioned the sensitivity, specificity and ROC. Index test and cutoff value for the marker have been calculated before the study only in one study done in saliva samples. However, the major shortcomings of these two studies[13,14] is that the clinical diagnosis of OLP and oral leukoplakia is not confirmed by tissue biopsy.

Table 2: Data extraction of saliva samples

Author and year	Samples	Mean±SD	*P*	Sensitivity (%)	Specificity (%)	Cutoff value
Fathi *et al*., 2013	Atrophic lichen planus Erosive lichen planus Reticular lichen planus	1.06±0.70 0.99±0.24 4.21±1.51	0.02	35.3	100	>1.25

Table 3: Data extraction of serum samples

Author and year	Samples	Mean±SD (ng/ml)	*P*	Sensitivity (%)	Specificity (%)	Cutoff value
Chang *et al*., 2013	Oral leukoplakia OSCC Control	296.5±208.7 473.5±447.4 126.1±100.7	OLP versus HC <0.001 OSCC versus HC <0.01	67.4	90	95th percentile of HC value

OSCC: Oral squamous cell carcinoma, OLP: Oral lichen planus, HC: Healthy control, SD: Standard deviation

Table 4: Data extraction in tissues

Author and year	Samples	Mean±SD	*P*	Sensitivity	Specificity	Cutoff value
Carvalhos *et al*., 2014	48 - OL 20 - OSCC 21 - HC	OL OSCC - 75% HC	OL versus OSCC *P*=0.014 OSCC versus HC *P*=0.014	Not mentioned	Not mentioned	Not mentioned
Chen *et al*., 2008	27 - OLP 15 - OSCC 11 - HC	Nonatrophic OLP - 60% Atrophic OLP - 91.67% OSCC - 93.33% HC - 9.1%	*P*=0.025	Not mentioned	Not mentioned	Not mentioned
Paulsova *et al*., 2012	OLP - 71 Control- 10	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Rajendran *et al*., 2006	20 - OSMF 20 - HC	Stromal expression of MMP-9 OSMF - 100% HC - 20% Epithelial expression of MMP-9 OSMF - 25% HC - 35%	Stromal expression of MMP-9 *P*=0.00 Epithelial expression of MMP-9 *P*=0.565	Not mentioned	Not mentioned	Not mentioned
Tortorici *et al*., 2008	170 - Oral leukoplakia 170 - Normal mucosa	Immunoexpression is stronger in leukoplakic tissue when compared to normal mucosa	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Al Rawi *et al*., 2014	28 - OLP 6 - OSCC 6 - HC	OLP - 2.5±3.06 OSCC - 23.17±6.67 HC - 1.5±0.54	0.00	Not mentioned	Not mentioned	Not mentioned

OSCC: Oral squamous cell carcinoma, OLP: Oral lichen planus, HC: Healthy control, OSMF: Oral submucous fibrosis, MMP-9: Matrix metalloproteinase-9, SD: Standard deviation

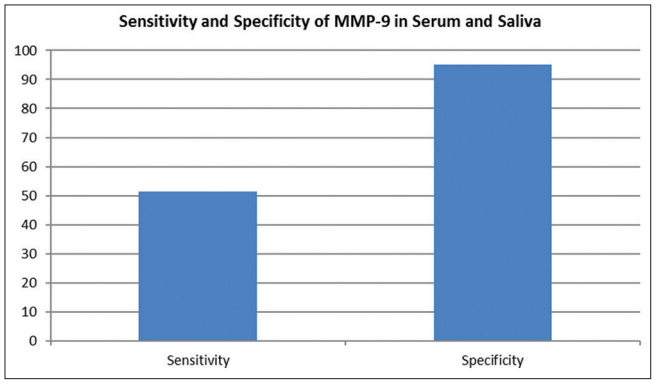

Figure 3: Sensitivity and specificity of matrix metalloproteinase-9 in detecting oral potentially malignant disorders

CONCLUSION

From these studies, the levels of MMP-9 in potentially malignant disorder shows elevation in the eight studies when compared to healthy control samples, but decreased levels than OSCC.[14,17,19] The studies were heterogenous and were done on different samples such as tissue serum and saliva. The expression of MMP-9 is in different scale of measurements in different studies. Hence, there is a need for homogenous studies with tissue, saliva and serum sample of same patient and longer follow-up periods. Six of the studies have been done in the tissue samples of OPMDs; there is only one study in serum and one study in noninvasive diagnostic tool such as saliva.

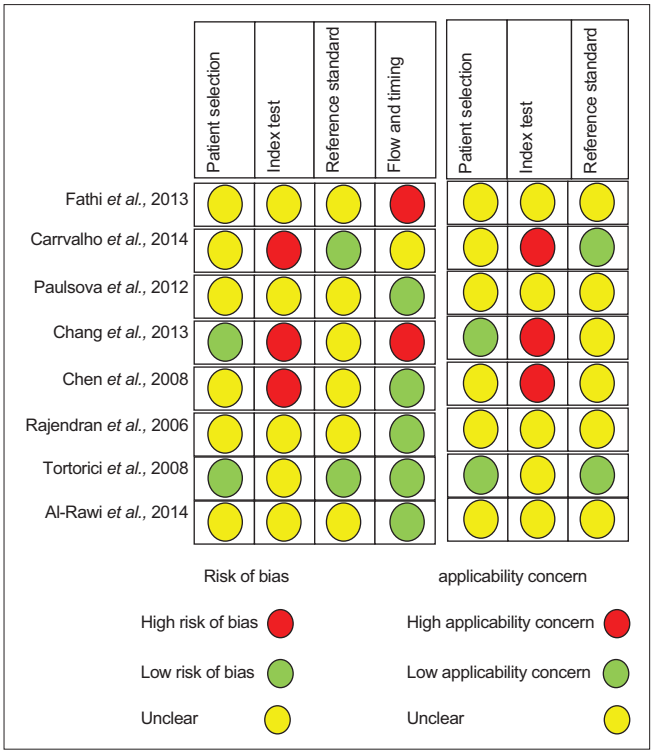

Figure 4: Risk of bias and applicability concern

Due to the technical difficulty in handling saliva and storing it without the degradation of the content, minimally invasive serum samples would be better in evaluating the MMP-9 and quantifying its expression in oral potentially malignant disorders such as oral leukoplakia, OLP and OSMF.

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Conflicts of interest

There are no conflicts of interest.

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