

## ORIGINAL ARTICLE

## ANALYTICAL STUDY OF SALIVARY MMP-12 EXPRESSION IN ORAL SUBMUCOUS FIBROSIS

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**Background:** Oral submucous fibrosis (OSF) is majorly a pathology pertaining to Asian population, reported most in Pakistan, India, Nepal, Bangladesh, and Taiwan. Equilibrium existing between Matrix metalloproteinases (MMP) & tissue inhibitors of matrix metalloproteinases is imperative for the normal integrity of connective tissues. However, this mechanism is disturbed in the presence of OSF, resulting in an increase in the extracellular matrix. **Methods:** It is an analytical study including two groups with a total of 60 participants. The first group consists of 30 healthy participants and the other group consists of 30 patients presenting with oral submucous fibrosis. Collected samples of saliva were stored at -80 °C after centrifugation. For ELISA investigation, the procedure was performed as per manufacturer's instruction. Salivary matrix metalloproteinases- 12 concentration was estimated with the help of a standard curve. Data was analysed using SPSS 23. Mann Whitney test was applied to determine the difference existing in Matrix metalloproteinases- 12 levels between healthy and oral submucous fibrosis participants.  $p$ -value <0.05 was contemplated as significant. **Results:** Statistical investigation indicated significant difference in Matrix metalloproteinases- 12 levels between Oral submucous fibrosis and healthy group ( $p < 0.05$ ). Saliva samples obtained from oral submucous fibrosis patients demonstrated raised concentrations of Matrix metalloproteinases- 12 as compared to healthy participants. **Conclusion:** Our study demonstrates significant upsurge in Matrix metalloproteinases- 12 expression in samples of saliva obtained from oral submucous fibrosis patients as compared to healthy individuals. Therefore, salivary Matrix metalloproteinases- 12 could serve as a useful diagnostic marker for OSF.

**Keywords:** Oral pathology; Oral submucous fibrosis; Matrix metalloproteinases; Saliva

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## INTRODUCTION

Oral submucous fibrosis (OSF) is majorly a pathology of Asian population.<sup>1</sup> It is most commonly reported in Pakistan, India, Nepal, Bangladesh and Taiwan.<sup>2,3</sup> According to several studies, the overall prevalence of OSF in India is reported between 0.03–3.2%.<sup>4</sup> In Pakistan, it's considered as a significant health problem owing to its potential of transforming into oral squamous cell carcinoma (3–19%), which is one of the most reported cancers in Pakistan.<sup>1,5,6</sup>

The most significant constituents favouring the development of OSF are water and ethanol soluble compounds.<sup>7</sup> Betel nut chewing is directly linked with the development of OSF.<sup>8,9</sup> It disturbs an individual's immune system, leading to decreased levels of TGF-beta & IFN- $\gamma$ .<sup>10,11</sup> Elevated levels of copper are present in betel nut and areca nut. The incidence of OSF rises as areca nut is consumed in combination with tobacco.<sup>8</sup> Role of superoxide dismutase and malondialdehyde has also been implicated in the pathogenesis of OSF. Levels of superoxide dismutase are diminished whereas malondialdehyde levels are elevated.<sup>8,12</sup>

Malnutrition associated with vitamin and protein deficiency pertains with the incidence of OSF. A close association has also been observed with serum iron, zinc and selenium deficiency.<sup>13,14</sup> Serum iron deficiency alters epithelial configuration and increases mucosal permeability which results in inhibiting barrier protection function. Selenium functions as an anti-fibrosis element and its deficiency results in reduced peroxidase activity which restrains the eradication of noxious constituents. Oral mucosa gets prone to fibrosis as external stimulation continues.<sup>15</sup>

Various molecular pathways, cytokines and cells take part in the pathogenesis of OSF. Aberration in the metabolism of collagen, epithelial-mesenchymal transition and myofibroblast differentiation, hypoxia etc. contribute to OSF development.<sup>15</sup> Equilibrium existing between MMPs & tissue inhibitors of MMPs (TIMPs) is fundamental for the maintenance of normal integrity of connective tissues. However, this mechanism is disturbed in the presence of OSF resulting in an increase in the extracellular matrix. This disturbance is expressed

as a reduction in collagen degradation and excessive fibrosis due to downregulation of MMPs and upregulation of TIMP.<sup>16</sup>

This analytical study aims to estimate MMP-12 levels in saliva among oral submucous fibrosis patients and compare with healthy participants.

## MATERIAL AND METHODS

This analytical study was performed at Oral and maxillofacial surgery department in Karachi, Pakistan. The sample size was computed by Pass software, version 11. The first group consists of 30 healthy participants and the other group consists of 30 patients who demonstrated clinical signs and symptoms of oral submucous fibrosis. OSF patient were confirmed and then selected for the study after clinically evaluating the signs of the disease such as blanching of mucosa, fibrosis and limitation of mouth opening and symptoms like decreased salivation, difficulty in mouth opening, difficulty in mastication. However, individuals demonstrating absence of clinical manifestation of pre-malignant condition were selected for healthy group. Patients reporting any chronic medical disease, malignancy, salivary gland disorder or autoimmune disease were excluded from the study.

Informed consent, which was according to Helsinki's declaration was obtained from each participant prior to obtaining the saliva sample. After obtaining consent, questionnaire was filled out. The Performa consisted of two parts. The first part consisted of sociodemographic data. The second part consisted of data pertaining to medical history and data related to features of oral submucous fibrosis.

Saliva was collected via passive drooling in a 15 ml falcon tube and then centrifuged (6000 xg) for 10 minutes at 4 °C. The supernatant was watchfully separated. The Eppendorf tubes were stored at -176 °F. For ELISA investigation, the tubes were thawed at room temperature. Specially designed kit (USCN- Wuhan USCN business Co., Ltd) was used and the procedure was performed as per manufacturer's instruction. With the help of a standard curve, salivary MMP-12 concentration was recorded.

Data was entered and analysed using SPSS 23. Mann Whitney test was applied to determine difference in MMP-12 concentration between healthy and OSF participants. *p*-value <.05 was contemplated as significant.

## RESULTS

Healthy group comprised of 30, out of which 5 (17%) were males and 25 (83%) were females. The mean

age was 28. OSF group consisted of 25 (83.3%) males and 5 females (16.6%). The mean age was 33. Around 16 (54%) patients belonging to OSF group reported betel nut as their oral habit, followed by 6 (21%) patients reporting tobacco smoking and 8 (25%) patients reporting smokeless tobacco as their oral habit. Among the healthy participants, around 20 (65%) participants reported with no oral habit. However, 4 (15%) participants reported tobacco smoking and 6 (20%) patients reported betel nut as their oral habit.

The most common associated sign and symptom among OSF patients was trismus, which was reported by 28 (96%) patients. Other symptoms and signs recorded were thickening of mucosa 28 (93%), oral pain 24 (80%), mouth dryness 23 (76%), burning sensation 21 (70%) and lips fibrosis 21 (40%).

Median MMP-12 concentration in saliva samples obtained from the two study groups was analysed. Statistically significant difference in median value was seen in MMP-12 concentration between healthy and OSF participants (*p*< .001). Patients presenting with OSF exhibited enhanced MMP-12 concentration as compared to healthy participants (Table-1)

**Table-1: Concentration of MMP-12 among controls and OSF (ng/ml)**

Study Participants	n= 60	Median (IQR)	<i>p</i> -value <sup>ü</sup>
Healthy	30	0.75 (0.57)	< 0.001*
OSF	30	12.1 (0.09)	

<sup>ü</sup> - *p*-value computed using Mann Whitney test  
IQR- Interquartile range

As per Passi staging for OSF<sup>17</sup>, majority of the OSF patients (53%) presented with grade III. However, 26% patients presented with grade IV and 20% with grade II. None of the study participants presented with grade I. Statistical results (Table-2) demonstrated non-significant difference in mean MMP-12 concentration between OSF stages (*p* >.05).

**Table-2: MMP-12 concentration among OSF stages (ng/ml)**

OSF stages	n=30	Mean (SD)	MSE ( <i>p</i> -value <sup>ë</sup> )
I	0	-	11.6 (0.991)
II	6	12.36 (4.1)	
III	16	12.58 (3.3)	
IV	8	12.56 (3.0)	

<sup>ë</sup> - *p*-value calculated using ANOVA  
MSE- Mean square error

## DISCUSSION

OSF is a potentially malignant condition of the oral region and pharynx characterized by collagen deposition in submucosa of the oral cavity.<sup>18,19</sup>

Owing to its potential to transform into head and neck squamous cell carcinoma (2–8%), diagnosis of OSF before its transformation may help reduce the health burden pertaining to SCC.<sup>20,21</sup>

MMPs cause disparity between synthesis and degradation mechanism of extracellular matrix.<sup>18</sup> MMPs and TIMPs appear to play a substantial role in maintaining accurate and balanced metabolism of collagen. This balanced is disturbed in OSF which results in enhanced extracellular matrix deposition.<sup>22</sup> Over the years, several studies have demonstrated the expression of salivary MMPs in OSF. The current study observed the concentration of salivary MMP-12 in OSF patients and compared the levels with healthy participants. The difference in MMP-12 concentration between the two groups was significant. Statistical analysis demonstrated increased MMP-12 concentration in OSF patients as compared to healthy participants.

In contrast to the increase in MMP-12 levels in OSF patients as per the results of the present study, past studies revealed that MMP-1 expression appears to diminish in OSF patients in comparison with normal oral mucosa.<sup>16,23</sup>

A study entitled, ‘molecular pathology of malignant transformation of oral submucous fibrosis’ reports MMP-1, MMP-2, MMP-9 as factors involved in OSF.<sup>24</sup> In another study, immunoreactivity of MMP-1, MMP-2 and MMP-9 was scored in both stromal and epithelial tissues. Statistically significant expression of all these MMPs was observed in stromal staining. However, non-significant results were observed in epithelial staining.<sup>25</sup> Elevated concentration of tissue inhibitors of MMP-1 and MMP-2 (TIMPs 1 and 2) have been observed in fibroblasts and are considered as initial markers of OSF and ageing.<sup>26</sup>

Similar to the results of the current study, which demonstrated increased MMP-12 concentration among OSF participants, evaluation of MMP-2 in OSF and its correlation with disease severity extrapolated increased expression as OSF advances and can be considered as a significant mediator in OSF progression and pathogenesis.<sup>27</sup> It was proposed in a study that this protease can assist in recognising the advancement of the condition and reveals its function in malignant transformation.<sup>27</sup> A similar study reports increased expression of MMP-2 along with TIMP-2 as OSF progresses to the advanced stage.<sup>28</sup> In contrast to this, a study demonstrated the role of arecoline in areca nut on MMP-2 expression and concluded that arecoline reduces the gelatinolytic activity of MMP-2 along with an elevated expression of TIMP-1 in human buccal mucosal fibroblasts (BMFs).<sup>29</sup> Also another study conducted in India concluded that single nucleotide polymorphism in MMP-2 and MMP-9

promoter region may not be related to susceptibility in OSF.<sup>18</sup>

As per Passi D, staging for OSF<sup>17</sup>, disease grade was noted for each patient. A major part of the group belonged to grade III. Statistical analysis revealed non-significant difference in salivary MMP-12 concentration between the OSF stages. Similarly, non-significant difference in MMP-1 concentration was reported among various histological grades of OSF.<sup>23</sup>

## CONCLUSION

Our study demonstrates significant expression of salivary MMP-12 in oral submucous fibrosis patients as compared to healthy individuals. Therefore, salivary MMP-12 could serve as a valuable diagnostic marker for oral submucous fibrosis.

### Conflict of interest:

None declared by the authors.

### Funding:

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## AUTHORS' CONTRIBUTION

ZS: The principal investigator, made a substantial contribution to the conception, design, data collection, sample processing and manuscript writeup. AHS: & UZ: supervision and contribution to data collection, sample processing and critical evaluation. SA: & MMM: contributed to sample collection, processing and drafting. ZS: & AK: Data analysis and interpretation.

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