Brief Report



# A distinct inflammatory and fibrogenic cytokine milieu in oral submucous fibrosis

**Roopa Yadahalli[1], Gargi S. Sarode[1], Namrata Sengupta[1], Dharmarajan Gopalakrishnan[2], Sachin C. Sarode[1]**

[1]Department of Oral Pathology and Microbiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India; [2]Department of Periodontology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India

*Correspondence to:* Dr. Namrata Sengupta, BDS, MDS. Department of Oral Pathology and Microbiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Sant Tukaram Nagar, Pimpri, Pune 411018, Maharashtra, India. Email: dr.namrata.sengupta@gmail.com.

**Abstract:** Oral submucous fibrosis (OSF) is regarded as a potentially life-threatening disease owing to strikingly high percentage of malignancy. The immune system has been a vital determining aspect for the disease progression and consequence. Fibrosis in OSF is usually preceded by infiltration of inflammatory leukocytes followed by release of various cytokines which trigger fibroblastic proliferation and deposition of excessive collagen fibers. Cytokines hence play a major role in regulation of the disease outcome. The assessment of these signalling proteins is essential in order to comprehend the disbalances that occur in the immune system with respect to OSF and its progression. Thus, in this study inflammatory and fibrogenic cytokine analysis was performed by employing flow cytometry in the biopsy specimens of OSF and control groups after institutional scientific and ethical clearance. A nonparametric paired *t*-test was carried out to determine the upregulation and downregulation of these molecules and their statistical significance. Some of the cytokines showed increase in their levels in a transient manner in different stages of OSF compared to control group. A few showed considerable downregulation in the late stage of OSF compared to early stage. The dynamic changes in the levels of the specific immune system molecules orchestrate the pathogenesis of OSF and subsequently aid in determination of therapeutic strategies for the treatment of the disease. The promising results observed in this study demands future studies in this direction.

**Keywords:** Fbrogenic cytokines; galectin-9; oral submucous fibrosis (OSF); pentraxin-3 (PTX3); plasminogen activator inhibitor-1 (PAI-1)

Received: 26 November 2022; Accepted: 19 July 2023; Published online: 21 August 2023.
doi: 10.21037/fomm-22-67
**View this article at:** https://dx.doi.org/10.21037/fomm-22-67

Oral submucous fibrosis (OSF) is regarded as a potentially life-threatening disease owing to strikingly high percentage of malignancy. The progression of this fibrotic disease is modulated largely by the action of the immune system. Fibrosis in OSF is usually preceded by infiltration of inflammatory leukocytes followed by release of various cytokines which trigger fibroblastic proliferation and deposition of excessive collagen fibers (1). Fibrogenic cytokines hence play a major role in regulation of the disease outcome. Cytokines such as interferon-alpha 2 (IFN-α2) participates in adaptive immune response while expression of tumour necrosis factor-alpha (TNF-α) promotes the migration of leukocytes into the microenvironment (2). Transforming growth factor-beta (TGF-β) takes part in collagen degradation pathway, wherein, increased levels of TGF-β correspond to a decreased collagenase activity (3). The assessment of these signalling proteins is essential in order to recapitulate the disbalances that occur in the immune system with respect to OSF and its progression. Thus, it is of utmost importance to understand the individual responses of these signalling molecules in the initiation and progression of OSF to ascertain the dynamics of the disease and lay a foundation for further studies.

To understand the role of cytokines such as IFN-α2, TNF-α, TGF-β (isoform 1 and 2), galectin-9, pentraxin-3 (PTX3), soluble form of CD40 ligand (sCD40L), soluble

**Table 1** Levels of cytokines in normal tissue, early and late stage of OSF tissue (pg/mL)

| Serial number | Chemokines | Normal tissue (pg/mL) | Early stage of OSF (pg/mL) | Late stage of OSF (pg/mL) | Levels |
|---|---|---|---|---|---|
| 1 | IFN-α2 | 6.443 | 7.090 | 9.445 | Upregulated |
| 2 | TNF-α | 20.554 | 30.221 | 49.099 | Upregulated |
| 3 | TGF-β | 10.223 | 77.055 | 98.022 | Upregulated |
| 4 | Galectin-9 | 9.043 | 23.334 | 27.212 | Upregulated |
| 5 | PTX3 | 6.004 | 54.032 | 75.011 | Upregulated |
| 6 | PAI-1 | 6.099 | 44.322 | 85.112 | Upregulated |
| 7 | sCD40L | 15.033 | 24.332 | 18.322 | Downregulated |
| 8 | sRAGE | 5.334 | 36.332 | 30.187 | Downregulated |
| 9 | sTREM1 | 7.443 | 76.332 | 43.098 | Downregulated |

OSF, oral submucous fibrosis; IFN-α2, interferon-alpha 2; TNF-α, tumour necrosis factor-alpha; TGF-β, transforming growth factor-beta; PTX3, pentraxin-3; PAI-1, plasminogen activator inhibitor-1; sCD40L, soluble form of CD40 ligand; sRAGE, soluble receptor for advanced glycation end products; sTREM1, soluble form of triggering receptor expressed on myeloid cells-1.

receptor for advanced glycation end products (sRAGE), plasminogen activator inhibitor-1 (PAI-1), and soluble form of triggering receptor expressed on myeloid cells-1 (sTREM-1), the analysis of these cytokines was carried out in OSF patients. A total of 21 clinically diagnosed OSF patients and 10 healthy control subjects were included and the cytokine analysis was performed by employing flow cytometry in the biopsy specimens of early and late stages of OSF (4) and also in the control group after institutional scientific and ethical clearance (No. DYPV/EC/102/18). Patients with stage 1 and 2 of OSF were considered in early stage while patients with stage 3 and 4 in late stage OSF. The values obtained from flow cytometry were in median fluorescence intensity (MFI) format and further converted to pg/mL. A nonparametric paired *t*-test was then carried out to determine the upregulation and downregulation of these molecules and their statistical significance.

To appreciate the immune dynamics of OSF it was very important to check the levels of these molecules with disease progression. In early-stage OSF no significant increase was observed in the levels of IFN-α2. Contrastingly in the late stage, there was a gradual but significant upregulation in its level. TGF-β and TNF-α also showed significant upregulation in the late stage. Similarly, the levels of galectin-9 were seen to be upregulated at the early stage due to increased inflammation and significantly upregulated in late stage OSF compared to control group. PTX3 showed hyper secretion in early stage of OSF to inhibit tumour response which leads to inflammation and in the late stage its level was upregulated even more since its main function is to promote tissue repair. The levels of PAI-1 were upregulated in both early and late stage in comparison to control group. sCD40L, sRAGE, and sTREM1 showed upregulation in early stage but contrastingly their levels downregulated in late stage (*Table 1*).

By assessing such molecules, we aim to build a detailed map of the landscape orchestrated by the dynamicity of these molecules which hold the crucial potential of determining the fate of the disease and consequently of the patient. IFN-α2 is an anti-fibrotic molecule which in our study showed slight upregulation in the late stage of OSF. This could be attributed to the presence of inflammation as it is pro-inflammatory or due to its anti-inflammatory levels trying to reduce the levels of increased inflammation and fibrosis. A vital anti-inflammatory molecule, TGF-β, is activated due to the presence of macrophages and other apoptotic bodies (5). As per our study results, it is clear that the innate immune system is activated thus resulting in inflammation. In order to curb the pro-inflammatory activities, TGF-β is released. Naturally the level of TGF-β is upregulated in both early stage and late stage of OSF which was found in our study also. Since TGF-β also essentially regulates the activity of alpha-smooth muscle actin (α-SMA) which is presented by the activated myofibroblasts, it can be derived that TGF-β essays a crucial part in the metabolism of collagen associated with fibrosis (6).

Active innate immune system and inflammation lead to the secretion of TNF-α by macrophages. The values of

TNF-α can be considered as one of early prognostic markers in the detection of immune havoc of OSF due to the transient increase in the values from early to the late stage. Also, with gradual progression of inflammation and immune response endothelial cells secrete galectin-9 which is capable of evading immune responses (7). In the current study, the levels of galectin-9 were seen to be upregulated in early and late stage relating it to increased inflammation. The elevated level of PAI-1 is usually noted in fibrotic tissues. The present study showed transient increase in PAI-1 and TGF-β in both the stages of OSF and values were higher in the late stage suggesting that inflammation and fibrosis in the late stage is high. High expression of PTX3 is known to induce epithelial-mesenchymal transition (EMT) and increase tumour growth and invasion (8). OSF is a potential condition to exhibit EMT and gradually progress to malignancy. Our study showed significant upregulation of PTX3 in late stage, which hints at possible EMT post-fibrosis. So, levels of PTX3 can act as a perfect marker to predict the transformation of OSF into oral cancer.

sCD40L is an ideal molecule to analyse the anti-tumour response. The interaction of fibroblasts and mast cells or T cells via CD40-CD154 signalling is serious for fibroblast initiation early in the sequence of fibrosis (9). sRAGE is a molecule actively participating in chronic inflammation. The upregulated levels of sRAGE in both the stages as observed in our study suggest its role in fibrosis and inflammation. sTREM1, which is usually expressed by innate immune cells can also be considered as an essential biomarker to analyse severity of disease (10).

Thus, this work reports on a study of the dynamic relationship of specific immune system mediators and the disease of OSF with each other. Classification of cellular markers based on their regulation and immune dynamics in the normal versus early and late-stage and early versus late-stage can be understood by this study. While some mediators showed increase in their levels in a transient manner, a few showed considerable downregulation in the late stage of OSF. The promising results observed in a small sample size demands future studies in this direction. Thus, by deriving details regarding the molecules studied in this work, we can harness their ability to not only serve as biomarkers for OSF but also can modulate them to employ as treatment for the amelioration of the disease.

## Acknowledgments

*Funding:* None.

## Footnote

*Data Sharing Statement:* Available at https://fomm.amegroups.com/article/view/10.21037/fomm-22-67/dss

*Peer Review File:* Available at https://fomm.amegroups.com/article/view/10.21037/fomm-22-67/prf

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://fomm.amegroups.com/article/view/10.21037/fomm-22-67/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional scientific and ethical committees of Dr. D. Y. Patil Vidyapeeth (No. DYPV/EC/102/18) and written informed consent was obtained from the subjects.



## References

1. Kovacs EJ, DiPietro LA. Fibrogenic cytokines and connective tissue production. FASEB J 1994;8:854-61.
2. Paul F, Pellegrini S, Uzé G. IFNA2: The prototypic human alpha interferon. Gene 2015;567:132-7.
3. Ankle MR, Kale AD, Nayak R. Mast cells are increased in leukoplakia, oral submucous fibrosis, oral lichen planus and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2007;11:18-22.
4. Ranganathan K, Mishra G. An overview of classification schemes for oral submucous fibrosis. J Oral Maxillofac Pathol 2006;10:55-8.
5. Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by

TGF-beta. J Biochem 2010;147:781-92.
6. Shih YH, Wang TH, Shieh TM, et al. Oral Submucous Fibrosis: A Review on Etiopathogenesis, Diagnosis, and Therapy. Int J Mol Sci 2019;20:2940.
7. Yasinska IM, Sakhnevych SS, Pavlova L, et al. The Tim-3-Galectin-9 Pathway and Its Regulatory Mechanisms in Human Breast Cancer. Front Immunol 2019;10:1594.
8. Wesley UV, Sutton I, Clark PA, et al. Enhanced expression of pentraxin-3 in glioblastoma cells correlates with increased invasion and IL8-VEGF signaling axis. Brain Res 2022;1776:147752.
9. Kawai M, Masuda A, Kuwana M. A CD40-CD154 interaction in tissue fibrosis. Arthritis Rheum 2008;58:3562-73.
10. Jolly L, Carrasco K, Salcedo-Magguilli M, et al. sTREM-1 is a specific biomarker of TREM-1 pathway activation. Cell Mol Immunol 2021;18:2054-6.

doi: 10.21037/fomm-22-67
**Cite this article as:** Yadahalli R, Sarode GS, Sengupta N, Gopalakrishnan D, Sarode SC. A distinct inflammatory and fibrogenic cytokine milieu in oral submucous fibrosis. Front Oral Maxillofac Med 2023.