Expression of Cartilage Oligomeric Matrix Protein in Primary Oral Submucous Fibrosis Cell line

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Abstract-

Introduction: Oral submucous fibrosis (OSMF) is a chronic disease that affects the oral cavity, characterized by inflammation, fibrosis, and loss of elasticity of the oral mucosa. Cartilage oligomeric matrix protein (COMP) is a non-collagenous extracellular matrix protein that has been shown to play a role in the development of fibrosis in various tissues, including the oral cavity. However, the expression of COMP in OSMF has not been well studied.

Objective: The objective of this study was to investigate the expression of COMP in a primary OSMF cell line in order to decipher its potential role in the development of OSMF.

Methods: Primary OSMF cell line were established from biopsy samples obtained from patients with OSMF. The expression of COMP was evaluated by reverse transcription-polymerase chain reaction (RT-PCR). The COMP levels between passage-2 (P-2) and passage-10 (P-10) were also assessed.

Results: RT-PCR revealed that COMP was significantly overexpressed in primary OSMF cell line compared to normal oral mucosa cell lines. The COMP levels between passage-2 (P-2) and passage-10 (P-10) also showed significant difference.

Conclusions: Our results reveal that COMP is significantly overexpressed in OSMF cell line. These findings suggest that COMP may play a role in the development of OSMF and can act as a potential therapeutic target for the treatment of the disease.

Keywords: Oral submucous fibrosis; Primary cell line; Cartilage Oligomeric Matrix Protein

I. Introduction

Oral submucous fibrosis (OSMF) is a chronic disease that affects the oral cavity, characterized by inflammation, fibrosis, and loss of elasticity of the oral mucosa. It is a progressive disease that can lead to difficulty in opening the mouth, as well as pain and burning sensations in the mouth.[1] OSMF is a major public health concern, particularly in India and other parts of Southeast Asia, where it is a leading cause of oral cancer. A significant increment in the prevalence of OSMF was recorded during the last four decades in India, from meagre 0.03% to 6.42%.[2] The clinical presentation of OSMF varies depending on the stage of the disease.[3] In the early stages, patients may experience burning sensations in the mouth and difficulty in opening the mouth. As the disease progresses, the oral mucosa becomes thickened and fibrotic, leading to trismus (limited opening of the mouth) and dysphagia (difficulty swallowing). In advanced cases, patients may develop oral cancer.[3]

The exact cause of OSMF is not well understood, but it is believed to be related to the use of areca nut, a substance commonly used in the preparation of betel quid. Areca nut is known to contain a number of carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons, which are thought to play a role in the development of OSMF.[4] According to an estimate, 10–20% of the total global population consumes areca nut in a variety of formulations.[5] A study by Tilakaratne et al. established a clear dose-dependent relationship between duration and frequency of chewing areca nut and development of OSMF.[6] Other risk factors for OSF include smoking, alcohol consumption, and a diet low in fruits and vegetables.[7] Different therapeutic and surgical treatment modalities for alleviating the symptoms of OSMF have been proposed, but no definitive treatment is contemporarily available.[8] The search for an effective treatment modality continues to this day thereby, making it dire necessity to decipher various other pathophysiological mechanisms to lay the groundwork for better therapeutic regimens in order to come up with definite curative treatment.[9]

Cartilage oligomeric matrix protein (COMP) is a noncollagenous, extracellular matrix protein that plays a role in the structural integrity of cartilage through its interaction with several extracellular matrix proteins, including type I, type II, type IX collagen, and fibronectin.[10] Primarily localized in cartilage, it is also considered as a marker of cartilage turnover.[11] Furthermore, it has also shown higher expression in areas of fibrotic scars and systemic sclerosis on skin, and have been speculated to have a role in vascular wall remodeling.[12] As OSMF primarily constitutes of fibrosis of juxta epithelium of oral mucosa therefore, a possibility exists that a relationship exhibiting higher expression of COMP in OSMF may be found.

As current modalities are unable to provide definite treatment therefore, significant results in COMP expression may help in establishing a possible new molecular target for OSMF. This may help in paving path for devising new and better drug therapies that may ultimately cure OSMF. In addition, the establishment of OSMF cell line can act as a screening platform for drug testing associated to OSMF in the future. Therefore, the aim of our study

was to assess the expression of COMP in primary OSMF cell line in order to establish an alternative pathway for OSMF in safe and convenient *in vitro* environment.

II. MATERIALS AND METHODS

The samples for establishment of primary cell line were obtained from Ziauddin Dental Hospital and laboratory work was performed at Multi-disciplinary Research Laboratory – I at Ziauddin Medical College, Clifton Campus. The ethical approval was obtained from Ethical Review Committee (ERC) at Ziaudin University (Reference code: 3820521MSOM). Informed consent was taken from the participants. Tissue was obtained from the non-inflamed posterior buccal mucosa during surgical extraction of the third molar in controls. On the other hand, biopsy of fibrotic areas in the buccal mucosa for the experimental group for establishing primary OSMF cell line.

Primary cell culture isolation was performed after obtaining buccal mucosa tissue from OSMF patient. Afterwards, enzymatic digestion was performed via trypsin and the sample was cultured in Dulbecco modified eagle medium (DMEM) supplemented with fetal bovine serum and penicillin/streptomycin. Oral fibroblasts were observed under the inverted microscope on the fifth day. On achieving 80% confluency, the fibroblasts were trypsinized and sub-cultured till 10 passage.

III. RESULTS

Expression of COMP in controls and OSMF fibroblasts showed significant difference. (p= >0.05). The comparison of COMP levels between controls and OSMF fibroblast in shown in Table 1. Likewise, on the comparison of different passages (P-2 and P-10) significant difference has also compared between the two passages as P-10 showed a significant increment when juxtaposed with P2. The gene expression of COMP at P-2 and P-10 is shown below in Figure 1.

COMP levels	Mean	Standard deviation	Significant difference	p-value
Controls	1.00	0.00	0.04*	<0.05
OSMF	48.35	19.45		

Table 1. Expression of COMP in controls and OSMF fibroblasts

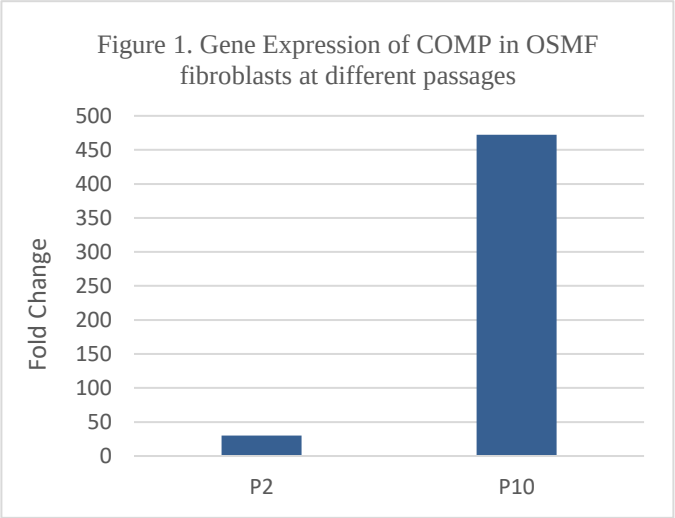
For the purpose of gene expression analysis, Trizole reagent was used to extract the RNA. For phase separation, 200ul of chloroform was utilized. It was followed by centrifugation at 2000 rpm for 8 minutes. Afterwards, Isopropanol precipitation was used to retrieve the RNA. It was centrifuged and air dried. The pallet were suspended in 20ul Nuclease-free water. The extraction of RNA was quantified using a Multi Scan Sky Spectrophotometer. The manufacturer's protocol was followed while synthesizing cDNA with the "Revert Aid First Strand cDNA synthesis Kit." Penicon's primer 3 software was used to design the primer sequence. GAPDH was taken as a housekeeping gene.

The primer sequence was as follows:

GAPDH; Forward 5′-CCAGAACATCATCCCTGCCT-3′
Reverse; 5′CCTGCTTCACCACCTTCTTG-3′
COMP; Forward 5′-CCAGGACGACTTTGATGCAG-3′
Reverse; 5′-TTGTCTGCACGATCTCCCTT-3'

RT-qPCR was utilized to assess COMP gene expression. To make a 20ul volume, cDNA and primer combination were mixed to SYBR green master solution. The steps of denaturation, annealing, and extension cycles were performed for 40 cycles. CT values were obtained for expression analysis.

Independent sample t test was used to calculate the difference between CT value of Control and OSMP cells. Post Hoc analysis was applied to identify significant difference between Passage-1 (P-1) and Passage-10 (P-10).



IV. DISCUSSION

Oral submucous fibrosis (OSMF) is a chronic, progressive, premalignant condition of the oral mucosa primarily characterized by the presence of juxta epithelial inflammation subsequently accompanied by fibroelastic alteration in the lamina propria and atrophic changes in the epithelial layers.[13] World Health Organization (WHO) has classified OSMF as an oral precancerous condition that exhibits high rate of malignant potential.[14] According to a study conducted in Karachi, a percentage of 26.60% was reported as malignant among the patients of OSMF.[15] A retrospective study carried out in China, concluded in its results

that oral squamous cell carcinoma (OSCC) evolving from OSMF was clinically more invasive and showed higher metastatic tendency and recurrence potential, as compared to OSCC with conventional origin.[16]

COMP has shown significantly higher expression in areas of fibrosis that includes liver, lung, kidney and pancreas. In 2016, in an experiment performed on murine rats, carbon tetrachloride (CCl4) injected Comp-/- mice showed less liver injury and fibrosis compared to the control mice. The results of the study showed that COMP contributed to liver fibrosis by regulating collagen deposition.[17] A year later, Andréasson et al. reported significant increase in COMP levels in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.[18] They further concluded that COMP had an upstream role in enhancing hepatic fibrosis. A study by Vuga et al. (2013) reported significant increment in COMP levels in idiopathic pulmonary fibrosis (IPF) patients as compared to controls.[19] In another study, after the immunohistochemical analysis of scleroderma (skin, lung, and kidney tissue) was conducted to detect COMP levels, it was concluded that COMP was significantly expressed in fibrotic areas of scleroderma compared to the non- fibrotic ones.[20]

A few studies have been reported on primary cell cultures of normal oral fibroblasts and fibroblasts from areca nut chewers that contributed significantly in deciphering the morphology and differentiation of fibroblasts *in vitro*.[21] In 2011, primary fibroblast cultures were established by the collagenase disaggregation technique and their phenotypic and growth characteristics were studied.[22] In 2017, an extensive laboratory study conducted by Banerjee et al., additionally assessed the response of the fibroblast cell lines to different concentrations of arecoline.[21] In 2019, Adtani et al. successfully performed a study aimed at the development of *in vitro* primary oral fibroblast to assess OSMF.[23] One new finding reported in the present study is the increment in COMP expression in different passages of OSMF cell line. In our study, we compared the COMP levels in P-2 and P-10 with each other and found significant increase in P-10 as compared to P-2 therefore, indicating that COMP levels in the OSMF fibroblasts increase in successive passages in a gradual manner.

The present study suggests that COMP may also provide diagnostic value as it can also help in early stage detection of OSMF. Our study was conducted to assess the expression of COMP in primary OSMF cell line in order to establish an alternative pathway for OSMF in safe and convenient *in vitro* environment. As current modalities are unable to provide definite treatment therefore, significant results in COMP expression may help in establishing a possible new molecular target for OSMF. This may help in paving path for devising new and better drug therapies that may ultimately cure OSMF. In addition, the establishment of OSMF cell line can act as a screening platform for drug testing associated to OSMF in the future.

V. CONCLUSION

Our results demonstrate that COMP is significantly overexpressed in OSMF cell line as compared to cells of normal mucosa and also shows considerable difference in different passages of OSMF. These findings suggest that COMP may play a role in the development of OSMF and may serve as a potential therapeutic target for the treatment of this disease. Further studies are needed to confirm these findings.

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