Evaluation of Myofibroblasts by Expression of Alpha Smooth Muscle Actin (α-SMA) in Oral Sub mucous Fibrosis

Dr. Mamatha GS Reddy1, Dr. Sachin Sarode2, Dr. Rajiv S Desai3
1Reader, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Vidyapeeth’s, Dr. D.Y. Patil Dental College and Hospital, Pimpri, Pune-18, Maharashtra, India
2Professor, Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Vidyapeeth’s, Dr. D.Y. Patil Dental College and Hospital, Pimpri, Pune-18, Maharashtra, India
3Professor and Head, Department of Oral Pathology and Microbiology, Nair Dental Hospital and College, Mumbai, Maharashtra, India

Abstract: Oral Sub mucous Fibrosis (OSF) is a chronic inflammatory oral potentially malignant disorder caused due to recant chewing characterized by excess collagen deposition in the submucosa leading to restricted mouth opening. Myofibroblasts are the contractile cells expressing α-SMA are seen in tissue repair and wound healing. They are also reported in various fibrotic conditions. The purpose of the study is to evaluate the expression of α-SMA in OSF. 10 normal and 25 OSF tissues are immunohistochemically stained for α-SMA. Majority of the cases in control group showed negative expression for α-SMA. The expression of α-SMA showed significant difference between control and OSF (p=0.00005), control and early OSF (p=0.006), control and advanced OSF (p=0.004). No significant expression was found between early OSF and advanced OSF. Myofibroblasts have a role in OSF. Any intervention that could prevent my fibroblastic differentiation can be therapeutic measure in OSF.

Keywords: Oral Sub mucous Fibrosis; Myofibroblasts; α-SMA.

INTRODUCTION

Oral sub mucous fibrosis (OSF) is an oral precancerous condition first described by Pindborg and Sirsat [1] characterized by chronic inflammation and a progressive fibrosis of the lamina propria and deeper connective tissues. It is considered as multifactorial disease with various etiological factors, areca nut being the most associated one [2-4]. The alkaloids of recant causes chronic irritation to the oral mucosa followed by chronic inflammation and release of various growth factors and inflammatory cytokines which leads to fibrosis [1,3].

Myofibroblasts are specialized fibroblasts expressing α-SMA possess the contractile property of smooth muscle cells. They have both physiological and pathological role like organogenesis, repair, inflammation and oncogenesis respectively. They secrete inflammatory and anti-inflammatory cytokines, lipid and gaseous inflammatory mediators, chemokines, growth factors as well as extracellular matrix proteins and proteases [5]. They are the predominant source of type I collagen and play a significant role in promoting ECM deposition. Differentiation of fibroblast into Myofibroblasts has been reported in fibrotic conditions. In tissue injuries, Myofibroblasts undergo apoptosis after normal healing, if persists due to stimulation by growth factors will progress to fibrosis [3,6]. Arecanut alkaloids have shown to increase α-SMA expression and my fibroblast differentiation from Buccal Mucosal Fibroblasts. Few studies have been reported the expression α-SMA in OSF. The aim of the study is to evaluate the α-SMA expression in various grades of OSF.

MATERIALS AND METHODS

The present study was carried out in Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune-18, after approval from institutional ethics committee. The study group comprised 25 sections of OSF blocks from the archives of Department of Oral Pathology and Microbiology. Clinical staging of the patients was made depending on mouth opening by Lai DR et al. [7] as Stage 1 (>35mm), stage 2 (30-35mm), stage 3 (20-30mm) and stage 4 (<20mm). The sections were stained for α-SMA by immunohistochemically method.

Immunohistochemistry

The tissue sections of 4µm thickness were deparaffinised and rehydrated in a graded series of alcohol. The sections were kept in pressure cooker at 90
degree for 10 min in citrate phosphate buffer (pH-6.0) for antigen retrieval. Wash with distilled water. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 min. α-SMA was applied as primary antibody for 45 min. at room temperature. After 2 washes with phosphate buffered saline, secondary antibody was applied to the sections; which are then incubated for 30 min. at room temperature. After wash with phosphate buffered saline dianinobenzidine was applied for 10 min. then the sections were counterstained with Mayer’s hematoxylin, dehydrated and mount.

The stained slides were interpreted as described by Soini et al. Scoring was based on (a) the intensity of the immunostaining in the lining epithelial cells (0 = absent, 1 = weak, 2 = moderate, 3 = strong, and 4 = very strong) and (b) the percentage of positive cells (0 = 0% positive cells, 1 = < 25% positive cells, 2 = 25-50% positive cells, 3 = 50-75% positive cells, and 4 = >75% positive cells). The final immunostaining score was determined by the sum of (a) and (b). Final scores ranged from 0 to 8 (0 = absent, 1-4 = weak, and 5-8 = strong). Staining of blood vessels was used as internal positive control.

**RESULTS**

The present study included 25 OSF cases and 10 controls, cases showed male predominance (M:F=20:5) and controls showed female predominance (M:F=4:6). Age group of OSF cases ranged from 16-55yrs with mean of 34.04yrs. Oral habits observed in cases were either gutka alone or in combination with betel nut and tobacco. Most of cases used gutka alone and belong to stage 3 & 4 there were 5 cases in stage 1, 8 cases in stage 2, 5 cases in stage 3 and 7 cases in stage 4. Analysis was done as early (Stage 1 and 2) and advanced (stage 3 and 4) stages of OSF. α-SMA were predominantly expressed by spindles located in the sub epithelium. Majority of the in control group showed negative expression for α-SMA (Fig 1). Strong expression was found in 7 out of 12 cases of early OSF (Fig 2). Strong expression was found in 4 and 9 out of 13 cases showed weak expression in advanced OSF (Fig 3). The expression of α-SMA showed significant differences between control and OSF (p=0.00005), control and early OSF (p=0.006), control and advanced OSF (p=0.004). No significant expression was found between early OSF and advanced OSF.(Table 1)

**Fig-1:** Negative α-smooth muscle actin (SMA) staining in controls. Internal positive control: Endothelial lining of the blood vessels demonstrating strong expression

**Fig-2:** α-Smooth Muscle Actin (α-SMA) staining in early OSF

Available online: [http://saspjournals.com/sjds](http://saspjournals.com/sjds)
Fig-3: α-Smooth Muscle Actin (α-SMA) staining in advanced OSF

Table-1: Comparison of α-SMA staining between control and different grades of OSF

<table>
<thead>
<tr>
<th>Marker</th>
<th>Groups</th>
<th>n</th>
<th>α-SMA staining</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Weak</td>
</tr>
<tr>
<td>α-SMA</td>
<td>Control</td>
<td>10</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>OSF</td>
<td>25</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Early OSF</td>
<td>12</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Advanced OSF</td>
<td>13</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Early OSF</td>
<td>12</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Advanced OSF</td>
<td>13</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

*significant

DISCUSSION

OSF is a progressive disease characterized by accumulation of ECM proteins and collagen deposition in the lamina propria followed by submucosa leading to stiffness of the mucosa and limited mouth opening [4]. Normally fibroblast contributes to connective tissue component by secreting collagen and non-collagen proteins. In fibrotic conditions, there is alteration of ECM and ratio of collagen deposition and degradation changes. One such condition is OSF, in which areca nut alkaloids causes persistent chemical and mechanical irritation to the oral mucosa and causes micro trauma that permits the diffusion of alkaloids into lamina propria eliciting an inflammatory response and release cytokines and growth factors like TGF-β1, a known key regulator of ECM assembly and remodeling[1,8,9]. TGF-β is involved in the differentiation of fibroblasts to Myofibroblasts, which is characterized by the presence of α-SMA containing stress fibres[10,11]. Physiologically they play a key role in growth and development of tissue or organ. In addition, they have role in tissue repair during wound healing. But as a response to tissue injury or inflammation, they secrete excessive extracellular matrix protein, in conditions such as scleroderma, hypertrophic scars, kidney, and lung and heart fibrosis [6].

Studies published have shown Myofibroblasts as the key cellular mediator of fibrosis in various fibrotic disorders [12]. The present study evaluated the expression of α-SMA in OSF tissues immunohistochemically and found significant difference results when compared with control (p=0.00005). These results were similar to the previously published reports [3,4,13]. Similarly, significant results were found when control group was compared with early OSF (p=0.006), and control with advanced OSF (p=0.004). The finding of increase number of Myofibroblasts in OSF shows failed wound healing due to lack of apoptosis of Myofibroblasts as mature Myofibroblasts disappear through apoptosis or by dedifferentiation after wound healing [6,8]. But no significance difference was found in α-SMA expression when early OSF was compared with advanced OSF (P=0.23). Previous studies by Angadi PV et al. Philip T et al, Gandhi P et al. have reported significance difference of α-SMA expression in early OSF and advanced OSF [3,4,14].

Different pathways have been studied and reported to understand the fibroblastic differentiation in OSF. Moutsim KA et al. showed the αvβ6-dependent activation of TGF-β1, which induced Myofibroblasts Trans differentiation from oral fibroblasts [15]. Chang YC et al. studied the arccosine induced myoepithelial differentiation in Buccal Mucosal Fibroblasts (BMF’s) of OSF tissues by investigating an EMT (Epithelial Mesenchymal Transition) transcriptional factor ZEB1[16]. The expression of Stage-specific embryonic antigen-4 (SSEA-4), a sialyl-glycolipid, cell surface marker for embryonic stem cells and pluripotent stem cells is demonstrated to increase in OSF tissues and arccosine-stimulated BMFs and play a role in my fibroblastic differentiation [17]. Presence Of fibrotic
lesions significantly increases the risk of cancer in many tissues. Myofibroblasts deriving from existing fibroblasts and also through EMT have been implicated in tumorigenesis [18]. Considering OSF as a fibrotic condition and evidence of expression of Myofibroblasts may show a possible role in malignant transformation.

CONCLUSION

The present results showed increase in expression of Myofibroblasts in OSF indicates its role in fibrosis. Understanding the role of Myofibroblasts in fibrosis and tumorigenesis, therapeutic measures against my fibroblastic differentiation could be used to prevent fibrosis and further malignant transformation.

ACKNOWLEDGEMENT

We would like to thank Dr. Pradnya Kakodkar, Department of Public Health Dentistry, Dr. D.Y. Patil Dental College and Hospital, Pimpri, Pune for her assistance in statistical analysis.

REFERENCES