ANNEXURES
Case History

DEPARTMENT OF ORAL PATHOLOGY AND MICROBIOLOGY

Principle Investigator- Dr. Sheetal Choudhari

<table>
<thead>
<tr>
<th>Sr. No.-</th>
<th>Date-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name-</td>
<td>Age/Sex-</td>
</tr>
<tr>
<td>Religion-</td>
<td>Occupation-</td>
</tr>
<tr>
<td>Address</td>
<td>Case paper no.-</td>
</tr>
<tr>
<td>Economic Status- Low/ Middle/ High</td>
<td>Contact no.-</td>
</tr>
<tr>
<td>Educational Status- Illiterate/ graduate/ non-graduate</td>
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<tr>
<td>Marital status-</td>
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A) Symptoms at first presentation-

<table>
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<tr>
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<th>Duration</th>
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<tbody>
<tr>
<td>1.</td>
<td>Burning sensation</td>
</tr>
<tr>
<td>2.</td>
<td>Pain &amp; discomfort from ulcers</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
</tr>
<tr>
<td>4.</td>
<td>Inability to open mouth</td>
</tr>
<tr>
<td>5.</td>
<td>Dysphagia</td>
</tr>
<tr>
<td>7.</td>
<td>Any other</td>
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<table>
<thead>
<tr>
<th></th>
<th>With hot &amp; spicy food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nil/ moderate/ severe recurrent/ continuous</td>
</tr>
<tr>
<td></td>
<td>Normal/ altered</td>
</tr>
<tr>
<td></td>
<td>Interincisal mouth opening</td>
</tr>
<tr>
<td></td>
<td>Yes/ no</td>
</tr>
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B) History-

1. Any systemic illness- DM/ TB/ Jaundice/ Anaemia/ Asthma/ HD/ Peptic ulcers/ Any other

2. Previous treatment

Drugs: Oral
Annexure I: Case History Proforma

Local
Systemic

3. Past dental history

C) **Family History**-

D) **Mental status**- Normal/ Average/ Psychic

E) **Personal history**-

a) Food habits:

   i. Type of regular diet: Veg./ Non veg./ Mixed

   ii. Consumption of spicy food

      - Occasional/ Regular/ Slight/ Moderate/ Severe

   iii. Green/ red chillies- Slight/ Moderate/ Severe

   iii. Fruits & green vegetables- Regular/ Occasional/ No

b) Relevant habits:

<table>
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<tr>
<th>Habits</th>
<th>Particulars</th>
<th>Frequency per day</th>
<th>Duration in years</th>
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<tr>
<td>Smoking</td>
<td>No habit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chewing</td>
<td>Areca only</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tobacco &amp; lime</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kharra (mava) (TLA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Betel quid with tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Betel quid without tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pan masala (brand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gutka (panmasala with tobacco)</td>
<td></td>
<td></td>
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Annexure I: Case History Proforma

<table>
<thead>
<tr>
<th>Combination of PM &amp; PMT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of kharra, PM, gutka</td>
<td></td>
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</tbody>
</table>

c) Average length of chew-
d) Teeth cleaning habit
e) Site of quid placement
f) Introduced by- Self/ Family/ Friends

F) **Clinical evaluation**
   a) IIO-
   b) Blanched mucosa (present/ absent)-
c) Appearance (marble like/ leathery/ muddy, pigmentation)-

d) Fibrous bands-

e) Tongue protrusion- i.Normal ii.Incisal tip
               iii.Up to lower lip iv.Beyond mucocutaneous junction
f) Burning Sensation Mild/ moderate/ severe
g) Oral hygiene
h) Gingival recession
i) Attrition- None/ Mild/ Moderate/ Severe

G) **Provisional Diagnosis**

H) **Clinical staging**

<table>
<thead>
<tr>
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<tr>
<td>Blanching:</td>
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<tr>
<td>Fibrous bands:</td>
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<tr>
<td>Ulceration:</td>
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Consent Form

NAME OF THE PARTICIPANT/PATIENT: -------------------------------------

TITLE OF THE RESEARCH PROJECT:

STUDY OF EXPRESSION OF FACTOR XIII A, MATRIX METALLOPROTEINASE-9 & VASCULAR ENDOTHELIAL GROWTH FACTOR IN ORAL SUBMUCOUS FIBROSIS

I, -------------------------------, the undersigned hereby authorize Dr.----------------------- to take oral biopsy for the diagnosis and contribution to the above mentioned research project. I also consent to the administration of local anesthesia which is necessary for taking biopsy.

I consent to the above mentioned procedure by my will and not by force or under fear. I hereby certify that I have fully understood the above authorization for the procedure. I have also been informed about the complications of the biopsy and local anesthesia. It has been informed to me that scarring may result as a complication during healing. I have also been assured about the required care/ treatment of the complications, if they occur.

I am aware of the benefits of this study to the society in terms of advancement of medical knowledge and therapeutic benefits to future patients.

I have been informed that the information from this study, if published in scientific journals or presented at scientific meetings, will not reveal my identity.
I hereby certify that I have decided to be the part of the study and have been informed about the study, the necessity of the procedure and its complications.

Patient’s Sign, Date and Place: -------------------------------------

Dentist’s Countersign, Date and Place: -------------------------------------
INVESTIGATOR CERTIFICATE

I certify that all the elements including the nature, purpose and possible risks of the above study as described in this consent document have been fully explained to the subject.

In my judgment, the participant possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate,

Signature of the Investigator: ________________ Dated:__________

Name of the Investigator: ___________________
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<th>Sr. No</th>
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<td>Mawa</td>
<td>II</td>
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<td>1</td>
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<td>II</td>
<td>Moderate</td>
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<td>45.78</td>
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Angiogenesis and Fibrogenesis in Oral Submucous Fibrosis: A Viewpoint

Sheetal S Choudhari, Deepak G Kulkarni, Sangeeta Patankar, Supriya M Kheur, Sachin C Sarode, Gargi S Sarode, Shankargouda Patil

ABSTRACT

Oral submucous fibrosis (OSF) is characterized by excessive fibrosis of submucosa. The degree of vascularity in OSF has always been a matter of debate. Angiogenesis is the key mechanism involved in regeneration and repair. It also plays an important role in various pathologic conditions. Angiogenesis may contribute to the progression of fibrosis in fibrotic disorders. Inhibition of pathological angiogenesis is considered to be a new strategy for the treatment of various fibrotic disorders. In OSF, angiogenesis can be related to progression fibrosis. This article briefly describes the role of angiogenesis in pathogenesis of fibrosis in OSF and the importance of inhibition of pathologic angiogenesis in its prevention and treatment.

Clinical significance: Understanding the association between angiogenesis and fibrogenesis can help in developing new therapeutic strategies for treatment of OSF.

Keywords: Angiogenesis, Fibrogenesis, Fibrosis, Hypoxia, Inflammation, Oral submucous fibrosis.

INTRODUCTION

Oral submucous fibrosis is characterized by juxtaepithelial inflammatory reaction followed by a generalized submucosal fibrosis. In OSF, the degree of vascularity/angiogenesis has always been a matter of debate. Studies on the mucosal vasculature in OSF have reported controversial results. According to the conventional concept, there is decreased vascularity in OSF which leads to epithelial atrophy of diseased mucosa. However, recent studies do not support the view of reduced vascularity in OSF. Further, it is still unclear whether angiogenesis can induce and occur in parallel with the progression of fibrosis in OSF.
Neutrophils and lymphocytes are involved in the early induction of angiogenesis by producing angiogenic factors, such as basic fibroblast growth factor and VEGF. Activated monocytes and macrophages can also induce angiogenesis. During the course of the disease, accumulation of inflammatory cells along with fibrosis can lead to hypoxia. In hypoxia, hypoxia-inducible factor 1 (HIF-1) induces angiogenesis and also stimulates inflammation through nuclear factor κB pathway. Newly formed vessels express various chemokines and adhesion molecules which stimulate recruitment of more and more inflammatory cells. This results in prolongation of inflammatory response. As newly formed vessels are immature and vulnerable, correction of the ischemic state of fibrosis could become difficult. This sets in persistent hypoxia resulting in the continuous production of proinflammatory and angiogenic molecules. These can eventually stimulate extracellular matrix (ECM) deposition and progression of fibrosis. Persistent hypoxic condition leads to elevation of HIF-1α protein levels which drives various factors related to fibrosis, such as transforming growth factor β1, thrombospondin-1, plasminogen activator inhibitor 1, and VEGF. Chronic hypoxia and consistent HIF-1 accumulation can potentiate the action of fibroblasts resulting in excessive matrix production through increased myofibroblastic differentiation. Thus, pathologic angiogenesis and hypoxia may act synergistically in disrupting normal tissue repair, resulting in development and progression of fibrosis.

Reduced vascular density along with diminished total vascular area and increased vessel obstruction is observed in advanced OSF. Reduced vascularity in advanced grade can be related to increasing matrix concentration, as increased matrix density can act as a physical barrier that restricts cell migration. Extracellular matrix stiffness has emerged as a critical extracellular parameter that can modulate capillary network formation and barrier integrity. Moreover, matrix stiffness can alter how endothelial cells respond to soluble, angiogenic factors released by stromal cells, such as VEGF. As more and more collagen is deposited in submucosa, fibrosis can impede material exchange, signal communication, and cell migration causing impaired angiogenesis with vessel loss resulting in reduced vascular surface area. This can explain reduced vascularity in advanced grade of OSF.

Angiogenesis can be related to fibrosis (Flow Chart 1). Pathologic angiogenesis should be intimately associated with fibrogenic progression in OSF as angiogenesis is a major feature of any wound-healing response, and chronic activation of wound healing is a general mechanism believed to be involved in the progression of fibrosis in OSF. Experimental evidence has suggested that the inhibition of angiogenesis can be used to prevent and treat liver and lung fibrosis. Mechanistic links between angiogenesis and fibrogenesis can provide novel clues for the development of antifibrotic therapeutic strategies in OSF. Clarity is required if impaired angiogenesis in OSF is a consequence of fibrosis due to angiostatic signals derived from the continuous deposition of ECM or if it is a cause for abnormal tissue repair and fibrosis due to impaired angiogenic signals. For this, angiostatic molecules should also be studied in OSF apart from studying angiogenic factors.

Based on aforementioned scientific background, one can hypothesize the presence of a vicious cycle between fibrosis and pathological angiogenesis in OSF. Hypoxia, which starts at an early stage in OSF, may contribute to the progression of this vicious cycle. It stimulates activation of HIF-1, which facilitates repair and revascularization by producing various growth factors and mediators. Although

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**Flow Chart 1: Interrelationship between angiogenesis and fibrogenesis**

1. Persistant area nut habit
2. Persistant tissue injury
3. Inflammation
   - Angiogenesis
     - Immature and permeable vessels
     - Failure to correct hypoxia
     - Continuous production of proinflammatory and angiogenic molecules
     - Stimulation of ECM products
8. Progression of fibrosis
required for successful repair, angiogenesis may fail to
correct tissue hypoxia as these newly formed vessels are
inefficient being more permeable and immature. Chronic
hypoxic condition causes continuous production of the
mediators that stimulate more ECM production leading to
progression of fibrosis. Thus, vascular remodeling and
hypoxia can be critically connected to the mechanisms of
fibrosis in OSF (Flow Chart 1). Initially, angiogenesis could
be a consequence of inflammation, rather than a cause
for disease initiation. However, at later stages, the contribution
of pathological angiogenesis may become more and more
causal resulting in progression of fibrosis, transition from
OSF to dysplasia, and then to squamous cell carcinoma.
Thus, pathologic angiogenesis along with inflammation and
hypoxia can exacerbate the severity of fibrosis in OSF.
Future studies are needed to further unravel the molecular,
cell, and tissue mechanisms linking angiogenesis and
fibrogenesis in OSF. Assessing the evidence supporting a
clear relationship between angiogenesis and fibrogenesis in
OSF would help in identifying new treatment targets for
OSF.

Clinical Significance

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   its pathogenesis and management. Br Dent J 1986
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   Characterisation and quantification of mucosal vascularisation in oral
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   quantification of mucosal vascularisation and its probable role in
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   and its comparison with oral squamous cell carcinoma. J Oral
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   inflammation and angiogenesis in the pathogenesis of primary
   moderate pterygium in a well-designed case-control study. Pak J Biol
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   modulating inflammation in the treatment of hyperglycemic wounds.
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   Botti P, Farkas L, Cho SX, Zepf JA, Azam T, et al. IL-32 promotes
10. Nagasaki T, Hara M, Nakamish H, Takahashi H, Sato M,
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    fibroblasts is critical for tumour angiogenesis: antinterleukin-6
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    endothelial growth factor in inflammatory processes. Postepy Hig
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    D, Beeck C, Hittmiya K, Fehberg D, Luedde T, et al. CCL2-dependent
    infiltrating macrophages promote angiogenesis in progressive liver
14. Novo E, Cannito S, Paternostro C, Bocca C, Mligietta A,
    Parola M. Cellular and molecular mechanisms in liver
15. Rosomordae O, Housset C. Hypoxia: a link between
    fibrogenesis, angiogenesis, and carcinogenesis in liver disease. Semin
16. Paternostro C, David E, Novo E, Parola M. Hypoxia,
    angiogenesis and liver fibrogenesis in the progression of chronic liver
17. Nath B, Szabo G. Hypoxia and hypoxia inducible factors:
    I, Van Vlierberghie H. Angiogenesis in chronic liver disease and its
19. Kukla M. Angiogenesis: a phenomenon which aggravates
    roles of angiogenesis in the induction of fibrogenesis and the
21. Distler JH, Jüngel A, Pile E, Mierla M, Michel BA,
    Gay RE, Kowal-Bielecka O, Mutsch-Cerinik M, Schett G, Marti HH,
    et al. Hypoxia-induced increase in the production of extracellular
    Dec;56(12):4203-4215.
    AP, Bertolami CN, Le AD. Mechanisms of hypoxia regulation of
    plasminogen activator inhibitor-1 gene expression in keloid
23. Lokmic Z, Musyoka J, Hewitson TD, Darby IA. Hypoxia
    and hypoxia signaling in tissue repair and fibrosis. Int Rev Cell Mol
    Biol 2012 Dec;296:139-185.
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    pathogenesis of primary liver fibrosis. Am J Physiol Cell Physiol
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    AP, Bertolami CN, Le AD. Mechanisms of hypoxia regulation of
    plasminogen activator inhibitor-1 gene expression in keloid
Is matrix stiffness a cause for malignant transformation of Oral Submucous Fibrosis?

Fibrosis is a pathological process characterized by mesenchymal cell infiltration and proliferation in the interstitial space, which occurs in order to repair epithelial injuries leading to the formation of granulation tissue. [1] The relationship between fibrosis and the development of carcinoma was described for the first time by Friedrich in 1939. [2] Since then, multiple studies have been attempted to determine the relationship between fibrosis and development of malignancy.

Fibrotic extracellular matrix (ECM) is found to be different biochemically as well as physically from normal matrix. Experiments have shown an increased differentiation of fibroblasts to myofibroblasts in presence of increased microenvironmental stiffness and tension. These newly activated cells continue synthesizing fibrotic matrix resulting in vicious cycle which can affect both stromal cells and epithelial cells in a continuously expanding “field effect.” Stiff ECM in fibrosis exerts more physical tension on the cells which transduces multiple intracellular signals, modifying cell behavior. Stiff ECM can alter cell growth, modify gene expression, and can alter cell differentiation. Moreover, ECM subjected to a higher physical tension induces structural modifications to ECM-associated molecules, modulating their bioactivity such as release of TGF-β. Matrix rigidity also regulates TGFβ-mediated epithelial-mesenchymal transition (EMT). [3] Distortion of tissue results from fibrosis, which may exert pathologically high levels...
of stretch on epithelial cells which may induce EMT. Fibrotic ECM may create conditions for cancer development by disrupting cell’s polarity and stimulating their proliferation. The extensive stromal components deposited could lead to hypovascularisation, activation of cancer cell proliferative programs, suppression of apoptosis, and increased cancer cell invasion and metastasis. [4] This can also compromise treatment efficacy, leading to increased patient mortality. Thus in chronic fibrosis, tissue stiffness results in a positive feedback which enhances the chronicity of fibrotic disease and can result in malignant transformation.

Oral submucous fibrosis (OSF), a chronic fibrotic condition, is characterized by stiffness of oral, pharyngeal and part of esophageal mucosa due to excessive deposition and cross linking of collagen. Fibrotic change in the submucosa in OSF is exhibited clinically as blanched appearance of the mucosa and palpation of fibrous bands traversing the oral mucosa causing rigidity and restricted movement. Histopathologically there is hyalinization of the submucosa due to deposition of dense matrix. This altered, stiff matrix in OSF can provide a fertile ground for malignant transformation. Mechanical stiffness can expand in a field like effect and can involve more and more adjacent tissue. Thus, conceptually, matrix stiffness as a cause of malignant transformation of OSF is quite conceivable. Studying mechanotransduction, mechanisms about cell contractility, and regulators of matrix rigidity can help in understanding the contribution of stiff matrix to the development of cancer. There is need to study the effect of extracellular mechanical stimuli on intracellular signaling pathways which can evoke cancer initiation in OSF. Effect of matrix stiffness on growth kinetics of cells, tension-dependent conformational alterations in integrin-associated proteins, crosstalk between integrins and RHO/ROCK (Ras Homolog Family Member/ Rho-associated protein kinase) pathway can be studied in relation to cancer initiation in OSF. In OSF reduced vascularity and decreased expression of matrix metalloprotienases is reported. However, angiogenesis and matrix invasion are important events in cancer initiation and progression. Mechanisms related to migration and invasion of malignant epithelial cells in stiff matrix could be different as there is limited space in stiff matrix. Endothelial cells are sensitive to the changes in physical properties of matrix. [5] Therefore, it will be interesting to know the role of matrix stiffness in alteration of angiogenesis and collagenases leading to cancer in OSF. In vitro models can be developed for studying the link between fibrosis and oral squamous cell carcinoma (OSCC). Finding the possible molecular links between stiff matrix in OSF and development of OSCC can help in designing treatment modalities.

There exist no definite protocols for the adoption of a particular treatment mode in OSF. Conventional treatment modalities for fibrosis in OSF in fact promote the loss of homeostatic equilibrium, which in turn can promote fibrosis. Therapies can be developed that can sense and respond to mechanical cues related to stiffness of stroma for treatment of OSF and OSCC associated with OSF. Mechanotransducers such as RHO/ROCK and targeting YAP/TAZ (yes-associated protein/Tafazzin) proteins, which are mechanotransduction effectors, can be explored as treatment targets. Mechanosensitive targeted therapies toward specific ECM components of stiff matrix and/or modifications to normalize the ECM at the stage of OSF could prevent the switch to protumourigenic/premetastatic environments. Therefore, increased matrix stiffness as a cause for malignant transformation in OSF needs an evaluation.

**Conflict of interest**
The authors indicated no potential conflicts of interest.

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