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

“The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter—for the future. His duty is to lay the foundation for those who are to come, and point the way.”
-Nikola Tesla

1. Introduction

Oral submucous fibrosis (OSMF) is an insidious chronic fibrotic disease and a well recognized premalignant condition that involves oral mucosa and occasionally pharynx and upper portion of esophagus\(^1\). Apart from oral cavity, fibrosis occurs in other parts of the body including liver, lung, kidney and skin\(^2-6\). These fibrosing conditions are listed by WHO among the top Global Burdens of diseases\(^7\). Despite of this global prevalence, there is little progress in development of effective anti-fibrotic treatment modalities. This could be because the exact pathogenesis of this stromal aberration with consequent deposition of fibrous tissue is still enigmatic to us. The pathogenesis of these fibrosing conditions including OSMF is multifaceted and incompletely understood. The characteristic features of fibrosis include increased differentiation and persistence of myofibroblasts, increased deposition of collagen and its decreased degradation. In OSMF fibrosis is initiated by injury to the oral mucosa because of chemical and mechanical irritation caused by areca nut habit. This is
followed by an inflammatory response, neovascularization, increased differentiation of myofibroblasts which results in increased deposition of collagen. This initiation of tissue repair changes are actually similar to that seen in initial phase of physiologic wound healing. Acute injury initiates fibrogenesis during wound healing and these changes are transient and reversible. However, in OSMF the wound healing process never commences. Chronic tissue injury, due to persistent areca nut habit and constituents of areca nut, changes the microenvironment from pro-healing to profibrotic. Chronic activation of the wound healing response ultimately results in fibrosis.

Being an archetype of pathological fibrosis having a relentless course of progression and irreversible nature, it is important to investigate every aspect of OMSF pathogenesis which could have treatment implications to help reduce the morbidity of this obscure fibrotic condition. An imbalance in the normal processes of synthesis and degradation of the extracellular matrix (ECM) leads to fibrosis. In OSMF, there is gross imbalance in ECM remodeling. Disruption of the equilibrium between production and degradation of collagen leads to extra deposition of ECM in OSMF. In OSMF, progressive fibrosis of the subepithelial connective tissue accounts for its clinical features. Deposition of collagen in the oral submucosa is the most important change seen in the initiation of this disorder. Factor XIIIa (FXIIIa) also known as fibrin stabilising factor apart from its important role in blood coagulation plays a crucial role in ECM organization in wound healing and tissue repair. It stimulates fibroblastic proliferation. It mediates cross linking of collagenous and non-collagenous matrix proteins. Studies have shown an association between FXIIIa and fibrosis in various fibrotic lesions. Increased collagen production being one of the
mechanisms involved in pathogenesis of OSMF, the role of FXIIIa needs to be evaluated in this collagen metabolic disorder.

Along with increased deposition of collagen, its decreased degradation results in progression of fibrosis in OSMF. Researchers have reported decreased degradation of collagen as another pathologic mechanism responsible for OSMF. Matrix metalloproteinses are a family of transmembrane proteins that efficiently breakdown the components of ECM and basement membrane. They play an important role in tissue remodeling by degrading ECM in health as well as in disease. MMPs comprise of a family of about 28 proteolytic enzymes which are classified according to their substrate specificity. **Matrix metalloproteinase-9** (MMP-9), a gelatinase, can degrade denatured collagen of all types. Few studies have reported its expression in OSMF, there is little information on its exact expression with the increasing grades of OSMF.

In this chronic fibrosing disorder, the degree of vascularity/angiogenesis has always been a matter of debate. Though according to characteristic histopathological features of OSMF and the conventional concept, there is reduced vascularity in the submucosa of OSMF, but recent studies by Rajendran et al. and Desai et al. do not support the view of reduced vascularity in OSMF. On the contrary, they suggest that vascularity/angiogenesis in OSMF may have an important role to play in malignant transformation of OSMF. In order to have a clear understanding about the vascularity in OSMF, we tried to investigate the expression of Vascular Endothelial Growth Factor (VEGF) in OSMF. **VEGF** is a potent angiogenic cytokine involved in angiogenesis. In health, VEGF causes angiogenesis during development and wound healing. Its expression is known to be induced by hypoxia. In OSMF, as fibrosis progresses, to cope up tissue hypoxia, as an adaptive response there could be increase
in angiogenic factors including VEGF. To solve this dilemma of vascularity in OSMF over traditional concept, we tried to evaluate expression of VEGF in OSMF.

In normal physiology, there is interplay of FXIIIa, MMP-9 and VEGF as seen in wound healing. FXIIIa is proangiogenic. It upregulates VEGF at wound site. It causes VEGFR-2 activation resulting in enhanced endothelial cell proliferation and survival. VEGF, a potent proangiogenic factor, induces release of MMP-9 which in turn regulates angiogenesis. Expression of MMP-9 is regulated by several cytokines and growth factors, important amongst which is vascular endothelial growth factor. MMPs produced by endothelial cells causes break down of basement membrane of endothelial cells and their release resulting in new vessel formation. VEGF modulates the production and activity of these proteins. MMP-9 causes release of VEGF and thus promotes angiogenesis. MMP-9 downregulates FXIIIa in normal physiology during fibrin clot formation. It causes proteolytic degradation of FXIII subunits in fibrin clot. FXIIIa downregulates MMPs thus stabilizing ECM at wound site. FXIII keeps a check on MMP-9 hyperactivation and promotes angiogenesis. Thus there is interplay of FXIIIa, MMP-9 and VEGF in physiology and various pathologies. Considering these facts, this study is designed to better understand the pathogenesis of OSMF by evaluating expression of FXIIIa, MMP-9 and VEGF in OSMF.
Research gap analysis

1. OSMF has relentless progression with functional impairment and potential for malignancy. Being an archetype of pathological fibrosis having a relentless course of progression and irreversible nature, it is important to investigate every aspect which could have treatment implications to help reduce the morbidity of this obscure fibrotic condition. Hence there is need to better understand the molecular events which may pave way to help in therapeutic intervention or preventing the advancement of disease.

2. FXIIIa plays role in collagen matrix formation in physiologic and pathologic conditions through fibroblastic proliferation and cross linking of collagen. Studies have shown an association of FXIIIa and fibrosis. Few studies have reported expression of FXIIIa in some oral fibrotic lesions but not in OSMF. In OSMF, as increased collagen production is one of the mechanisms involved in pathogenesis of OSMF, the role of FXIIIa needs to be evaluated in this collagen metabolic disorder.

3. Matrix metalloproteinases (MMPs) are the enzymes, responsible for degradation of components of extracellular matrix. MMP-9 can degrade denatured collagen of all types. As found through standard databases MMP-9 expression is not yet studied in all grades of OSMF. Hence, there is need to study its expression with the increasing grades of OSMF.

4. There is controversial data on vascularity in OSMF. Medline search in English literature has found very few studies on angiogenesis in OSMF, which have yielded controversial results. Therefore there is need to evaluate angiogenesis in OSMF by using immunohistochemical marker VEGF for further objective elucidation.
5. Role of FXIIIa in lung and liver fibrosis has been proved and Anti-FXIIIa treatment modalities are being tried in these pathologies. Hence, the role of FXIIIa needs an elucidation in OSMF so as to design possible therapeutic interventions in OSMF based on role of FXIIIa.

**Research question**

1. Can expression of FXIIIa, MMP-9 and VEGF play role in pathogenesis in oral submucous fibrosis?
2. Does expression of these markers alter with the increasing grades of oral submucous fibrosis?
3. Can expression of FXIIIa be related with fibrosis in oral submucous fibrosis?

**Hypothesis**

- **Working Hypothesis**
  1. Altered expression of Factor XIIIa(FXIIIa), Matrix metalloproteinase-9(MMP-9) & Vascular endothelial growth factor (VEGF) in OSMF may play role in its pathogenesis.
  2. Expression of FXIIIa can be related with initiation of fibrosis in OSMF.

- **Null Hypothesis**
  1. There may not be any change in expression of FXIIIa, MMP-9 & VEGF in OSMF.
  2. Expression of FXIIIa may not be related with initiation of fibrosis in OSMF.
Anticipated Translatory Component

We thought this study anticipate that -

1. OSMF has relentless progression with functional impairment and potential for malignancy. Study may have an implication on a population of patients having OSMF.

2. Understanding the molecular events by studying the expression of FXIIIa, MMP-9 and VEGF may help in therapeutic intervention or preventing the advancement of disease.

3. If role of FXIIIa is related with fibrosis in OSMF, studies can be designed in future to test the therapeutic benefit of AntiFXIIIa in treatment of OSMF, as it is being tried in lung and liver fibrosis.

4. By studying the expression of MMP-9, future studies can be carried out using therapies based on MMPs in treatment of OSMF.