

Cytokines in Oral Submucous Fibrosis: A Bird's Eye View

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ABSTRACT

Introduction: Oral Submucous Fibrosis (OSF) is a chronic fibrosis condition of oral cavity. It is a multifactorial condition, arecanut chewing being the prime etiologic factors.

Objectives: Arecanut alkaloids stimulate the oral tissue initiating the inflammatory response. As an inflammatory response, cells release cytokines activating the cascade of different fibrinogenic pathways leading to fibrosis.

Conclusion: This review briefs about the various cytokines studied in Oral Submucous Fibrosis.

Key words: Chemokines; cytokines; fibrosis; interleukins; oral submucous fibrosis

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INTRODUCTION

Oral submucous fibrosis (OSF), first described as a potentially malignant disease in early 1950s shows preponderance in the Asian population. It is a chronic disorder, progressive in nature with its clinical presentation depending mainly on the stage of the disease at detection. Most of the patient's experience burning sensation in the mouth mainly on consumption of hot and spicy food, rigidity of circumoral muscles and tongue thereby inducing varying degrees of limitation in mouth opening and tongue movements. Several etiological factors have been proposed in the pathogenesis of OSF. Arecanut is considered to be the most important of all. Areca nut is mainly composed of alkaloids and flavonoids of which alkaloids are biologically key constituents. Arecoline, arecaine, guvacine, and guvacoline are the alkaloids that have been identified in areca nut. Arecoline is the most important constituent among others and has crucial part to play in the pathogenesis of OSF by causing an abnormal increase in the co-synthesis of collagen. Flavonoids in arecanut include tannins and catechins. These components are reported to directly affect the collagen metabolism.¹

Continuous exposure of the oral mucosa to betel quid leads to absorption of the quid ingredients that is the alkaloids and the flavanoids into the mucosa and its subsequent metabolism. These components together with their metabolites form a constant source of irritation to the oral mucosa. In addition to the irritation chemically, the coarse fibres in the betel quid causes mechanical irritation. The continuous friction from coarse fibres of arecanut results into micro trauma that in turn facilitates diffusion of the betel quid components alkaloids and flavanoids into the subepithelial connective tissue, giving rise to juxtaepithelial inflammatory cell infiltration. Habit persistence leads to chronic inflammation, which is characterized by the

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presence of activated T cells, macrophages, etc. A number of inflammatory mediators like prostaglandins are synthesized, which are secreted by the oral keratinocytes in response to areca nut extract has been reported.^{1,2}

The occurrence of cancer and tissue fibrosis has been substantially expedited by an abnormal and constant tissue inflammation. Therefore, betel quid ingredient induced oral mucosal inflammation can be deemed as a crucial event in OSF pathogenesis. Various cytokines like interleukin 6, tumour necrosis factor (TNF), interferon α , etc and growth factors like TGF- β are produced at the site of inflammation.²

Cytokines are low molecular weight (less than 30 kDa <200 amino acids) glycoproteins or pleiotropic proteins synthesized by various cell types like leukocytes which induces inflammation, haematopoiesis and aids in immune regulation.¹ They are classified based on their origin from either from Th1 cells or Th2 cells. Th1 cells produce IL-2, TNF- α and IFN- γ and Th2 cells produce IL-4, IL-5, IL 6, IL-9 and IL-

13. Of late, a new Th cell subset, Th17 and T regulatory cells (Treg) have been categorized with a distinct cytokine profiling unlike Th1 and Th2 cells, Th17 cells synthesize IL-17, IL-17F, IL-22 and IL25 and Treg cells type 1 (Tr1) predominantly secretes IL-10 and IFN- γ . It secretes IL-5 in lesser amounts and very low amounts of TGF- β and IL-2. Th3 cells (or Tr3), a subset of Treg mainly secretes TGF- β and in minimal amounts produces IL-10.³ In many of the pathological conditions like inflammation, angiogenesis, tumorigenesis, neurobiology, viral pathogenesis, fibrotic conditions, immune mediated diseases etc. cytokines have been studied extensively. OSF a chronic fibrotic oral disease has also demonstrated involvement of some cytokines. The alkaloid component in arecanut (arecoline and arecaidine), is known to induce activity of a number of inflammatory cytokines and growth factors in fibrotic conditions, especially OSF where the main etiological factor is arecanut chewing.⁴

Interleukin 1 (IL-1)

Interleukin 1 α and interleukin 1 β (IL-1 α and 1 β) are cytokines produced by macrophages, monocytes, fibroblasts and dendritic cells. It is also expressed by B lymphocytes, NK cells, microglia and epithelial cells. They are involved in the regulation of immune responses, inflammatory reactions, and hematopoiesis.^{5,6} Interleukin 1 contributes and promotes fibrosis in organs like skin and lungs.^{7,8} The expression of IL-1 α and IL-1 β in OSF were first demonstrated in OSF by Haque et al.^{4,9} Both the types were increased in the OSF tissues. Interleukin-1 β did not show much significance in OSF tissues and also in saliva of OSF patients.¹⁰

Interleukin 6 (IL-6)

IL-6 is a pro-inflammatory cytokine produced by activated immune cells and stromal cells, including T cells, monocytes/macrophages, endothelial cells, fibroblasts, and hepatocytes. Its involvement is seen in the pathogenesis of fibrotic diseases, including pulmonary and hepatic fibrosis and systemic sclerosis (SSc).¹¹ Production of IL-6 was more by the cultured buccal fibroblasts from OSF tissues after exposing them to betel nut alkaloids, arecoline or arecaidine.^{12,13} Elevated levels of IL-6 in serum and saliva is found in OSF patients.^{14,15} Significant expression of IL-6 was found by Haque et al in OSF tissues using Immunohistochemistry but not by the Rangaswamy et al study.^{4,16} Synthesis of IL 6 by peripheral blood mononuclear cells of OSF patients was demonstrated by Haque et al.⁹ Presence of IL 6 in saliva, serum and tissues of OSF suggests its role in the pathogenesis.

Interleukin 8 (IL-8)

IL-8 a chemokine produced by various cells like monocytes, neutrophils, epithelial, fibroblast, endothelial, mesothelial, and tumour cells. It is chemotactic to fibroblasts and stimulates collagen production during healing of wound. It also stimulates fibroblasts to produce α -smooth muscle actin.¹⁷ Increased levels of IL-8 was found in saliva of OSF patients.¹⁸ Haque et al demonstrated increased synthesis of IL-8 by the peripheral blood mononuclear cells. These studies could proof the role of IL 8 in OSF.⁹

Interleukin (IL-13)

Profibrotic cytokine IL-13 is produced by variety of cells like T helper type 2 (Th2) cells, mast cell, basophil cells, eosinophil

cells, etc.¹⁹ Various fibrotic conditions of liver, skin and lungs demonstrated role of IL-13.^{20,21} Resident fibroblast and recruited fibrocytes differentiate into myofibroblast in presence of IL-13.²² IL-13 is shown to be expressed in early stages of OSF suggesting its pathogenic role since the disease initiation.²³

Monocyte chemoattractant protein-1 (CCL2)

CCL-2 is a member of chemotactic cytokine chemokine family which are implicated in the initiation and progression of several physiological as well as disease processes. In the recent times, various studies have been deeply carried out to assess its role in activation of fibroblasts and angiogenesis in pathogenesis of fibrotic diseases.²⁴ Expression of CCL-2 in the OSF tissues could suggest its role in recruiting myofibroblasts in OSF.²⁵

Transforming Growth Factor β (TGF- β)

In OSMF, excess deposition of the extra cellular matrix is mediated by TGF β . It increases the collagen synthesis and decreases the collagen degradation.² TGF β has been fairly studied in OSF demonstrating its pivot role in OSF. TGF β is shown to be increased in OSF tissues.⁴ All the three isoforms of TGF β including the receptors TGF- β R1 and TGF- β R2II were shown to be expressed in OSF.²⁶ OSF shows upregulation of TGF β 1.^{27,28} TGF β 1 stained predominantly in early stage of the disease.^{23,29,30} TGF beta 1 mRNA was expressed by the keratinocytes of OSF epithelium.³¹ Progressive increase of TGF β 1 and TGF β 2 expression with the grades of OSF was shown by Kamat et al.^{32,33} Epithelial cells treated with areca nut extract secrete TGF- β which activates fibroblasts to deposit collagen.³⁴ Khan et al demonstrated activation of TGF- β through p-SMAD2 signalling pathway. Some of the TGF- β pathway genes were shown to be maximum in OSF tissues.²⁸ OSF changes were observed histologically with upregulation of TGF β 1 in a rat model after injection of arecanut and pan masala extracts.³⁵

Tumour necrosis factor -alpha (TNF- α)

Tumour necrosis factor -alpha regulates inflammation and also regulates transcription of collagen and collagenase.^{36,37} Genetic polymorphism of TNF- α has been demonstrated in OSF.³⁸ Increased levels of TNF- α in OSF patients suggests its role in pathogenesis.^{9,39,40} Increased TNF- α in OSF patients could also play a role in its malignant transformation.^{41,42}

Interferons

Three forms of Interferons IFN- α , IFN- β , IFN- γ have been demonstrated to inhibit the synthesis of collagen. IFN- γ targets various cells involved in collagen synthesis like dermal fibroblasts, human synovial fibroblasts like cells, human chondrocytes, rat myofibroblasts.⁴³ IFN- α , IFN- β , IFN- γ all of the cytokines are less or not expressed in OSF tissues and peripheral blood mononuclear cells (Haque 1998,2000). IFN- γ inhibits the collagen synthesis by OSF fibroblasts hence reverses the condition of OSF by intralesional injections.⁴⁴

CONCLUSION

Cytokines play an important role in the pathogenesis of fibrosis. Many of the members from cytokine family have been studied in the various fibrotic conditions. Very few of the cytokines have been explored in OSF. Understanding the pathogenesis of these cytokines through different pathways may help in the treatment of the disease.



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