

## Review Article

# Histopathological Changes in Oral Submucous Fibrosis: Updated Review

Santosh RP<sup>1\*</sup>, Maragathavalli G<sup>2</sup>, Ramesh DNSV<sup>3</sup>, Tejaswi K<sup>4</sup>, Ankita S<sup>5</sup> and Alam MK<sup>6</sup>

<sup>1</sup>PhD Scholar, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, India

<sup>2</sup>Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, India

<sup>3</sup>Department of Oral Medicine and Radiology, AMES Dental College and Hospital, India

<sup>4</sup>Department of Oral Medicine and Radiology, SVS Institute of Dental Sciences Mahabnagar, India

<sup>5</sup>Department of Oral Medicine and Radiology, New Horizon Dental College and Research Institute, India

<sup>6</sup>Department of Orthodontics, College of Dentistry, Jouf University, Kingdom of Saudi Arabia

\*Corresponding author: Santosh R Patil, PhD Scholar, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India

Received: June 16, 2022; Accepted: July 18, 2022;

Published: July 25, 2022

## Introduction

Oral submucous fibrosis (OSMF) is a chronic disease of insidious onset of unknown etiology affecting the oral and oropharyngeal region characterized by progressive fibrosis of the oral submucosa [1].

The most important clinicopathological aspects of OSMF are fibrosis and hyalinization of subepithelial tissue, which have a significant impact on the patients' quality of life. Myofibroblasts' presence and persistent expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) are regarded indicators of increasing fibrosis, and they are hypothesised to create a shift in the OSMF milieu, which leads to carcinogenesis. The current acknowledged pathogenic factor for OSMF is areca nut, despite the fact that the specific aetiology and pathophysiology have yet to be fully explained. Meanwhile, recent years have seen significant advancements in OSMF-related diagnostics and therapeutic therapy [2].

Various histopathological changes seen in OSMF patients varies according to the severity of the disease, in mild cases there is atrophic changes in epithelium which manifests as burning sensation. As the disease progress there is fibrotic changes in the connective tissue along with the changes in the muscles, this leads to limitation of mouth opening. In severe cases involvement of palatal and paratubal muscles leads to eustachian tube dysfunction in OSMF individuals.

Clinicians benefit from a comprehensive awareness of OSMF research advancement since it not only broadens their horizons but also gives fresh insights into OSMF research and therapy.

Since this condition is associated with oral epithelial dysplasia and cancer, OSMF is considered a premalignant condition. OSMF is regarded as a disease of collagen metabolic disorder showing increased

## Abstract

Oral submucous fibrosis is a collagen metabolic condition characterised by excessive collagen formation and reduced collagen breakdown. According to the studies, in addition to increased collagen build-up, there was also an increase in the level of muscle injury. The muscle change might be a symptom of the disease, with atrophy occurring as a result of the muscles' reduced functional activity caused by fibrosis, or it could be a necessary aspect of the disease's progression. As a result, histological alterations in OSMF patients will aid in future disease treatment interventions. The objective of the review is to consolidate the various histopathological changes seen in oral submucous fibrosis reported in literature.

Various electronic databases like Google Scholar, EMBASE, PubMed, SCOPUS, and Web of Science were searched for articles published until March 2022. Keywords, oral submucous fibrosis, histology, pathology, dysplasia, atrophy were used to search the data base.

**Keywords:** Oral Submucous Fibrosis; Histopathology; Dysplasia; Atrophy

collagen production along with decreased collagen degradation [1].

Various histopathological changes along with muscle involvement in the fibrosis process have previously been described in the literature. Atrophy, degeneration, and fibre necrosis have all been reported in varying degrees of muscle involvement in different stages of OSMF [2]. These muscular alterations might be a symptom of OSMF, with atrophy occurring as a result of the muscles' reduced functional activity caused by fibrosis, or they could be a vital consequence of the disease's course.

## Histopathological Features

### Epithelium

J. P Caniff [3] studied histological sections of thirty patients with OSMF, in which 87% of the patients had an atrophic epithelium, of which 27% were reported as having a flattened epidermal/demial junction. None of them had any evidence of epithelial hyperplasia, 33% of patients had non-keratinized or poorly keratinized epithelium, but in the remaining 67% of the cases, epithelium showed keratinized metaplasia, 13% had parakeratinization and 23% of patients had hyper orthokeratinization.

### Connective Tissue

Pindborg JJ [1] has described the connective tissue changes in four consecutive stages of OSMF as follows:

**Very early stage:** it is characterized by finely fibrillar collagen dispersed with marked edema. The inflammatory cells, mainly polymorphs with occasional eosinophils, are present.

**Early stage:** the juxtaepithelial area showed early hyalinization. The collagen is still seen as separated bundles, which are thickened

in young fibroblasts and are present in moderate numbers. The inflammatory cells are mostly mononuclear lymphocytes, eosinophils, and occasional plasma cells.

**Moderately advanced stage:** collagen is moderately hyalinized and amorphous changes start from the juxtaepithelial basement membrane.

Occasionally, thickened collagen bundles are still seen separated by slight residual edema. The cells present are mostly adult fibroblasts with elongated, spindle shaped nuclei and scanty cytoplasm. Blood vessels are constricted as a result of increased surrounding fibrous tissue. The inflammatory exudate consists of lymphocytes and plasma cells, although occasional eosinophils may be present.

**Advanced stage:** the collagen is completely hyalinized and is seen as a smooth sheet with no separate bundles. Edema is absent, the hyalinized area is devoid of fibroblasts although a thin elongated cell or vestigial nucleus is seen in a rare integral fibre bundle. Blood vessels are completely obliterated or narrowed. The inflammatory cells mainly seen are lymphocytes and plasma cells.

**J. P Caniff** [3] in a histological study of 30 oral submucous fibrosis patients, showed an accumulation of collagen beneath the basement membrane in all cases. 27% of patients had significant hyalinization of the excess collagen. Few had a chronic inflammatory cell infiltrate within the lamina propria, consisting of lymphocytes, plasma cells, monocytes, and macrophages.

### Epithelial Atypia in Oral Submucous Fibrosis

In histological study of J. P Caniff [3] of 30 oral submucous fibrosis patients, 27% had mild atypia within the epithelium, 7% showed mild to moderate changes and 3% of patients had marked atypia.

**Van Wyk CW** [4] compared the electron-microscopic features of 11 oral submucous fibrosis patients in moderately advanced and advanced stages with control specimens and noticed densely packed bundles of collagen in the lamina propria, reaching close to the epithelial connective tissue junction, to the blood vessel walls, salivary glands and muscle fibres.

**EI-Labban and Caniff** [2] investigated ultrastructural muscle changes in 2 groups of patients having oral submucous fibrosis. Nine punch biopsies were taken from the buccal mucosa of 9 patients with clinical submucous fibrosis in which blanching of the cheeks was observed and fibrous bands were palpable. Only 5 of these biopsies were enough to include muscle tissue. Two of the biopsies containing muscle were from patients with no restriction of movement, of the other three cases, two had severe limitation of mouth opening, and one was moderately affected. The majority of muscle fibres from the first group seemed normal electron microscopically, with just a few muscle fibres displaying a buildup of homogenous material and compression of the sarcomeres near to this material. Patients with rearmed mouth opening, on the other hand, had significant alterations and necrosis in a large proportion of muscle fibres. The plasma membrane of necrotic muscle fibres had totally disappeared, but the contour was preserved by an intact basal lamina. This research suggests that in submucous fibrosis, mouth opening may be limited not only by subepithelial fibrosis but also by the amount of degeneration.

**Rooban T** [5] studied 33 oral submucous fibrosis patients and divided into three grades on the basis of mouth opening. grade I (>40mm), grade II (20-39mm), and grade III (<19mm). Incisional biopsy from the right buccal mucosa was taken and histopathologically fibrosis was further graded as:

**Stage I:** Fibrosis limiting to the lamina propria.

**Stage II:** Fibrosis involving the superficial region of the muscle bundle.

**Stage III:** Fibrosis involving deeper regions of the muscle bundle.

**Stage IV:** Fibrosis replacing the muscle bundle.

According to mouth opening, 26.67% were in grade I oral submucous fibrosis, 66.67% in grade II oral submucous fibrosis, and 6.67% in grade III oral submucous fibrosis. 23.34% belonged to stage I fibrosis, 40% to stage II, 10% to stage III, and 26.67% to stage IV. When mouth opening was compared with the stage of fibrosis, then 42.9% of cases in stage I fibrosis belonged to grade I oral submucous fibrosis and 42.9% to grade II, 83.3% of cases in stage II fibrosis belonged to grade II oral submucous fibrosis whereas 66.7% of stage III fibrosis and 62.5% of stage IV to grade II oral submucous fibrosis.

**Hiroko Utsunomiya et al.**, [6] examined biopsy specimens of 40 oral submucous fibrosis patients and 10 biopsy specimens from normal buccal mucosa for the expression/deposition modes of 8 extracellular molecules by biochemistry, immunohistochemistry and in situ hybridization. They categorized the OSMF patients as following:

- **Early stage:** tenascin, perlecan, fibronectin, and collagen type III were characteristically enhanced in the lamina propria and the submucosal layer.
- **Intermediate stage:** tenascin, Perlecan, fibronectin, collagen type III, and etastin were extensively and irregularly deposited around muscle fibres.
- **Advanced stage:** extracellular matrix depositions decreased and were entirely replaced with collagen type I.
- Restricted mouth opening may be the result of the loss of a variety of extracellular matrix molecules including elastin with the homogeneity of collagen type I replacing muscle fibres.

**Kiran Kumar K** [7] in a hospital based study conducted on 72 oral submucous fibrosis patients correlated the clinical stage of the disease in terms of mouth opening with the histopathologic grading. Out of the 57 cases which were in clinical stage II, 91.2% had histological grading of I and II in equal proportions and 8.8% had histological grade III. Out of the 13 cases that showed clinical stage III, 52% showed histological grade II, 40% grade III, and 8% grade I.

### Muscle Involvement in OSMF

Masticatory muscle involvement and replacement with fibrous tissue were noted in individuals with OSMF. The exact and extent of fibrosis and its role in causing trismus are determined by several factors including the anatomical and physiological integrity of the underlying musculature [6].

Varying degrees of muscle changes including atrophy,

degeneration, and necrosis of fibres were noted as OSMF advance. A progressive decrease in the distance of muscle fibers from the epithelial surface was observed. Focal and complete loss of myofilaments and myofibrils and replaced amorphous material nucleus internalization along with thickened and dense collagen fibres interspersed with muscle fibres and extensive degeneration of muscle fibres were observed in advanced cases of OSMF. All these degenerative muscular changes are irreversible [2,9].

One of the earliest reports on muscle involvement in OSMF was by Binnie and Cawson [10], they reported a homogenous collagenous subepithelial zone along the degeneration of muscle fibres. Based on histopathologic observation Khanna and Andrade [11] reported thickened and dense collagen fibres interspersed with muscle fibres in moderately advanced cases of OSMF with an interincisal distance of 15-25mm and they also observed extensive degeneration of muscle fibres in advanced cases with interincisal distance of <15mm.

In a histopathologic study, Rooban [5] observed fibrosis extending into the superficial and deeper regions without visible changes in the muscle bundles. They also observed thin collagen fibres present in the muscle epithelium that showed broken sarcolemma. Along with this, fibrosis and hyalinization were also seen extending into the muscle bundle zone resulting in atrophy of the muscles. Apart from these, a few remnants of muscle fibres were seen and the missing muscle bundle area was replaced by fibrous tissue. Marked fibrosis areas with hyaline changes extending from subepithelial muscle layers and atrophic degenerative changes in the muscle fibres was reported by Utsunomiya [6].

Previous histopathological studies have revealed that muscle fibers undergoing necrosis are frequently delimited by edematous fluid and extravasated RBCs [2]. An explanation for edema surrounding the muscle fiber and subsequent muscle fibrosis in OSMF was stumbled upon during the literature search [9]. Chewing of areca nut it is known to induce stretch-induced muscle damage and activates stress-gated cation channels [12-14]. Which in turn allows (a) influx of sodium ions into the muscle cells, which leads to edema of the muscle cells and necrosis [12]; and (b) influx of calcium ions into the muscle cells uncoupling the mitochondria, resulting in an elevation in reactive oxygen species levels. This further activates the stress-gated cation channels, forming a positive feedback loop. Additionally, myofiber necrosis and subsequent inflammation also activate these channels, establishing a positive feedback loop. In fact, elevated intracellular calcium ion level have been shown to facilitate muscle cell degeneration and necrosis in OSMF [12].

Pathologic changes in the blood vessels conspire to reduce perfusion in the affected muscles, which results in ischemia and necrosis of the muscles [2]. The diminution in tissue perfusion can be demonstrated by ultrasonography as a reduction in flow and peak systolic velocity of blood [15]. This reduced perfusion of the muscles and their continued muscular activity can cause the further depletion of glycogen, which results in fibrosis.

Normally, injured muscle initiates the repair of myofibers via proliferation of satellite cells [5,16], which then form the myoblasts, and these differentiate into myocytes. The fusion of myocytes controls the formation of myofibers and the completion of muscle repair. However, both TGF- $\beta$ 1 and myostatin dedifferentiate these

cells into myofibroblast thereby contributing to muscle fibrosis. The conditional expression of TGF- $\beta$ 1 in muscle causes fibrosis and atrophy of muscle [17].

Increased muscular activity due to constant chewing of areca nut in the setting of reduced blood supply to the muscle causes glycogen depletion and forces the muscle to undergo anaerobic glycolysis, resulting in the production of pyruvic acid. The release of HIF-1 $\alpha$  due to reduced blood supply causes an increase in lactate dehydrogenase (LDH)-5, which in turn catalyzes the conversion of pyruvate to lactate. Lactate has been shown to activate latent TGF- $\beta$ 1 [18,19], which further increases HIF-1 $\alpha$  [19], forming a potential feed-forward loop. Increased TGF- $\beta$ 1 then drives myofibroblast differentiation. Experimental upregulation HIF-1 $\alpha$  and LDH-5 synergize with low doses of TGF- $\beta$ 1 in inducing myofibroblast differentiation. Additionally, inhibition of HIF-1 $\alpha$  and LDH-5 inhibits myofibroblast differentiation [18]. Moreover, lactate has also been shown to act on the collagen promoters, thereby directly promoting collagen synthesis [12]. Constant chewing of areca nut leads to muscle fatigue with a subsequent elevation in glycolytic activity [20]. Enhanced glycolytic activity leads to accumulation of pyruvate due to muscle hypoxia, which further leads to upregulation of LDH in muscle tissue. Furthermore, the hypoxic state associated with OSMF itself may also augment the LDH levels [20,21].

## Histopathological Changes in Palatal and Paratubal Muscles

Gupta et al., assessed the histopathological changes in palatal and paratubal muscles in OSMF patients. Incisional biopsy was obtained from the soft palate in clinically diagnosed cases of OSMF. In majority of the individuals, signs of chronic inflammation and fibrosis were observed in the submucosa. Dysplastic changes were also noted in some of the patients. Degenerative changes in palatal/paratubal muscles were found in the form of loss of cross striations. Oedematous muscle fibres were also seen along with atrophic changes. The authors concluded that there was definite involvement of palatal and paratubal muscles in OSMF and this may be the cause of eustachian tube dysfunction in OSMF individuals [22].

## Conclusion

Varying degrees of histopathological changes and involvement of muscle fibers are noted as OSMF progresses. Significant muscle damage in OSMF demonstrates that restricted mouth opening appears to be dependent not only on subepithelial fibrosis but also on the level of muscle damage, with extensive degeneration being the most conspicuous characteristic seen various studies. Thus, alterations in muscle fibres such as degeneration and necrosis, as well as subepithelial fibrosis, play a part in the pathophysiology of OSMF, which reveals clinically as trismus.

The common histopathological features of OSMF includes chronic inflammation, increased collagen deposition in the submucosal connective tissue of the oral mucosa, local inflammation in the lamina propria or deep connective tissue, and degenerative changes involving the muscles. Mucosal edoema alterations in the subepithelial area of the mucosa corresponding to the upper lamina propria were present at first, along with diffuse lymphocyte infiltration, but no major fibrotic changes had developed. In the middle stage, fibrosis had

progressed to the muscle layer, and transparency in the hypodermic region had begun to develop. There are significant patches of fibrosis in the late stage, with alterations from the subepithelial layer to the muscle layer. Collagen fibres have a heterogeneous orientation in early oral submucosal fibrosis, but a parallel direction in the late stage, and the microvessel density decreases dramatically from early to late.

## References

1. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral surgery, oral medicine, and oral pathology*. 1966; 22: 764-779.
2. El-Labban NG, Caniff JP. Ultrastructural findings of muscle degeneration in oral submucous fibrosis. *J Oral Pathol*. 1985; 14: 709–17.
3. Canniff JP, Harvey W, Harris M. Oral submucous fibrosis: its pathogenesis and management. *British Dental Journal*. 1986; 160: 429-434.
4. Wyk CWV, Seedat HA, Phillips VM. Collagen in submucous fibrosis: an electron-microscopic study. *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 1990; 19: 182-187.
5. Rooban T, Saraswathi TR, Zainab FHIA, Devi U, Eligabeth J, Ranganathan K. A light microscopic study of fibrosis involving muscle in oral submucous fibrosis. *Indian journal of dental research : official publication of Indian Society for Dental Research*. 2005; 16: 131.
6. Utsunomiya H., Tilakaratne WM, Oshiro K. Extracellular matrix remodelling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J Oral Pathol Med*. 2005; 34: 498–507.
7. Kumar KK, Saraswathi TR, Ranganathan K, Devi MU, Elizabeth J. Oral submucous fibrosis: a clinico-histopathological study in Chennai. *Indian journal of dental research: official publication of Indian Society for Dental Research*. 2007; 18: 106.
8. Sumathi M, Balaji N, Malathi N. A prospective transmission electron microscopic study of muscle status in oral submucous fibrosis along with retrospective analysis of 80 cases of oral submucous fibrosis. *Journal of Oral and Maxillofacial Pathology: JOMFP*. 2012; 16: 318.
9. Chawla H, Urs A, Augustine J, Kumar P. Characterization of muscle alteration in oral submucous fibrosis-seeking new evidence. *Medicina Oral, Patología Oral y Cirugía Bucal*. 2015; 20: e670-e677.
10. Binnie WH, Cawson RA. A New Ultrastructural Finding in Oral Submucous Fibrosis. *British Journal of Dermatology*. 1972; 86: 286-290.
11. Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management. Report of 100 cases. *International journal of oral and maxillofacial surgery*. 1995; 24: 433-439.
12. Klingler W, Jurkat-Rott K, Lehmann-Horn F, Schleip R. The role of fibrosis in Duchenne muscular dystrophy. *Acta Myologica*. 2012; 31: 184-195.
13. Yeung EW, Allen DG. Stretch-Activated Channels in Stretch-Induced Muscle Damage: Role in Muscular Dystrophy. *Clinical and Experimental Pharmacology and Physiology*. 2004; 31: 551-556.
14. Allen DG. Skeletal Muscle Function: Role of Ionic Changes in Fatigue, Damage and Disease. *Clinical and Experimental Pharmacology and Physiology*. 2004; 31: 485-493.
15. Manjunath K, Rajaram PC, Saraswathi TR, Sivapathasundharam B, Sabarinath B, Koteeswaran D, et al. Evaluation of oral submucous fibrosis using ultrasonographic technique: a new diagnostic tool. *Indian journal of dental research: official publication of Indian Society for Dental Research*. 2011; 22: 530.
16. Nichlani SS, Jagade MV, Ganeshan A. Benefit of using muscle relaxants in the routine treatment protocol of oral submucosal fibrosis: a pilot study. *Indian J Otolaryngol Head Neck Surg*. 2011; 63: 317–20.
17. Narola J, Pandey SN, Glick A, Chen Y. Conditional Expression of TGF- $\beta$ 1 in Skeletal Muscles Causes Endomysial Fibrosis and Myofibers Atrophy. *PLoS ONE*. 2013; 8: e79356.
18. Kottmann RM, Kulkarni AA, Smolnycki KA, Lyda E, Dahanayake T, Salibi R, et al. Lactic acid is elevated in idiopathic pulmonary fibrosis and induces myofibroblast differentiation via pH-dependent activation of transforming growth factor- $\beta$ . *American journal of respiratory and critical care medicine*. 2012; 186: 740-751.
19. Tuder RM, Lara AR, Thannickal VJ. Lactate, a novel trigger of transforming growth factor- $\beta$  activation in idiopathic pulmonary fibrosis. *American journal of respiratory and critical care medicine*. 2012; 186: 701-703.
20. Sivaramakrishnan M, Sivapathasundharam B, Jananni M. Evaluation of lactate dehydrogenase enzyme activity in saliva and serum of oral submucous fibrosis patients. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 2015; 44: 449-452.
21. Shetty SR, Chadha R, Babu S, Kumari S, Bhat S, Achalli S. Salivary lactate dehydrogenase levels in oral leukoplakia and oral squamous cell carcinoma: a biochemical and clinicopathological study. *Journal of cancer research and therapeutics*. 2012; 8: S123-5.
22. Gupta SC, Khanna S, Singh M, Singh PA. Histological changes to palatal and paratubal muscles in oral submucous fibrosis. *The Journal of Laryngology & Otolaryngology*. 2000; 114: 947-950.