

# A Study on Orientation of Collagen Fibres in Oral Submucous Fibrosis

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**Abstract-** Oral submucous fibrosis is a chronic debilitating disease of oral mucosa and is characterized by generalized fibrosis of the oral soft tissues which tends to present itself clinically as palpable vertical fibrous bands. Traditionally, stains such as van- Geison and various forms of trichrome have been used to detect collagen fibres in tissues. Thick and thin collagen fibres play a vital role in maintaining connective tissue integrity. However, both these stains fail to reveal thin collagen fibres. A special stain- picosirius red stain (PSR) that stained both thin and thick collagen fibres was used in this study. Thus, the present study is to demonstrate the orientation and color of collagen fibres in different grades of oral sub mucous fibrosis and normal mucosa and observed under polarized microscope. However, correlation to the grading of OSF will help in determining the changes in the connective tissue which precedes the neoplastic change in epithelium.

## I. INTRODUCTION

Oral submucous fibrosis is an insidious chronic disease affecting any part of the oral cavity and sometimes pharynx<sup>1</sup> and a well recognized potentially malignant condition of the oral cavity characterized by inflammation and a progressive fibrosis of the lamina propria and deeper connective tissue<sup>2</sup>. This condition was described first by Schwartz (1952) while examining five Indian women from Kenya, to which he ascribed the descriptive term “atrophia idiopathica mucosae oris”. later in 1953, Joshi from Mumbai redesignated the condition as oral mucous fibrosis, implying predominantly its histological nature.<sup>3</sup> Other names for this condition are “diffuse oral sub mucous fibrosis, idiopathic scleroderma of the mouth, idiopathic palatal fibrosis, sclerosing stomatitis, and juxta- epithelial fibrosis.”<sup>4</sup> The WHO definition for an oral precancerous condition- “a generalized pathological state of the oral mucosa associated with a significantly increased risk of cancer,” accords well with the characteristics of the OSF.<sup>3</sup> Epidemiological studies show 7% - 13% of OSF transforming into malignancy. The precancerous nature was first mentioned by paymaster who observed development of squamous cell carcinoma in one –third of his OSF patients.<sup>1</sup> oral submucous fibrosis is preceded by symptoms like burning sensation of the oral mucosa, ulceration and pain. The characteristic feature of oral submucous fibrosis is reduced movement and depapillation of the tongue, blanching and leathery texture of oral mucosa and progressive reduction of mouth opening.<sup>4</sup>

The histopathologic grading based on the criteria, which were modified from the original criteria given by Pindborg and Sirsat as given below:

**Grade 1:** Early hyalinization of juxtaepithelial area, plump young fibroblasts, dilated or congested blood vessels, and presence of inflammatory cells mostly mononuclear lymphocytes, eosinophils and occasional plasma cells.

**Grade 2:** Moderately hyalinized collagen, less marked fibroblastic response, presence of mostly fibrocytes, constricted blood vessels and inflammatory exudates consists of mostly lymphocytes and plasma cells with occasional eosinophils.

**Grade 3:** Complete hyalinization of collagen with depletion of fibroblasts in those areas, blood vessels are completely obliterated or narrowed and inflammatory cells are lymphocytes and plasma cells.<sup>5</sup>

One of the clinical symptoms of OSF is trismus, a limitation of mouth opening, this may eventually impair the ability to eat, speak and dental care may be difficult.

Connective tissue consists for the most part of collagen fibres. Collagen provides connective tissue with a unique combination of flexibility and tensile strength but is not very elastic (Junqueira et al. 1993)<sup>6</sup>. Collagen fibres play a vital role not only in maintaining structural integrity, but also in determining tissue function<sup>7</sup>. Regulation is necessary, not only to control the amount of collagen produced but also to control the fibre architecture. The synthesis of collagen is influenced by a variety of mediators including growth factors, hormones, cytokines and lymphokines. A prominent mediator is the TGF- $\beta$  which stimulates the production of collagens and other matrix components.<sup>8</sup>

In OSF, connective tissue changes are characterized by a deposition of dense collagen fibres. Even though collagen could be stained by various dyes, picosirius red stain was found to be more superior to other stains. It stained finer collagen fibres more intensely and increased their birefringence spectacularly.<sup>9</sup> Picosirius red special stain was used because the Sirius red, a strong cationic dye, stains collagen by reaching via its sulfonic acid groups with basic groups present in the collagen molecules. Collagen molecules, being rich in basic amino acids, strongly react with acidic dyes. Sirius red is an elongated dye molecule which reacts with collagen and promotes an enhancement of its normal birefringence due to the fact that many dye molecule are

aligned parallel with the long axis of the each collagen molecule.<sup>10</sup>

Further an attempt was made to correlate changes in orientation and color of collagen fibres with OSMF and normal mucosa.<sup>8</sup>

## II. MATERIAL AND METHODS

Total study group was comprised of 18 paraffin embedded archival blocks of OSF and 10 paraffin embedded archival blocks of normal mucosa as control group. The age group ranging from 17 to 40 years.

From each of the paraffin embedded tissue block, two sections of 5 µm thickness were prepared. One section were stained with haematoxylin and eosin stain, the other with picrosirius red stain. The picrosirius red stained sections were examined using polarizing microscope and the thickness of collagen fibres and their orientation with respect to the epithelium was assessed.

The thickness of the collagen fibres were examined by using Leica research microscope under polarized light and the orientation and thickness of the fibres was assessed.

The thickness of collagen fibres are measured using a micrometer eye piece.

## III. RESULTS

Our study revealed the results as follows: In mild cases of OSF, we examined loosely arranged thin collagen fibres in yellowish orange color. In moderate cases of OSF, we examined both thick and thin collagen fibres, where thin fibres showing, yellowish orange color and thick fibres showing, red colored fibres. In advanced cases of OSF, we observed tightly packed bundles of collagen fibres in red color and most of the fibres were seen parallel to the epithelium. In control group, the orientation of collagen fibres were haphazardly arranged with respect to the epithelium

### Evaluation using polarizing microscope

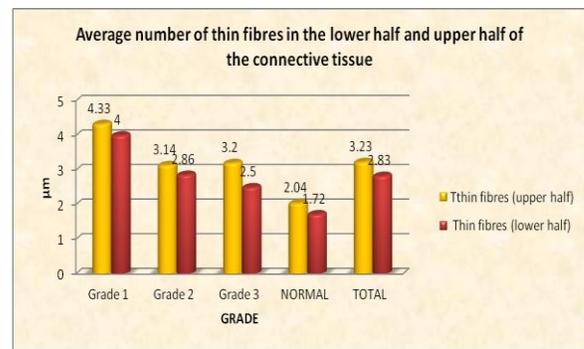
We evaluated polarizing colors of collagen fibres in OSMF and compared with those of control group. In mild case of OSF, the polarizing color of thin collagen fibres is yellowish orange in color. In moderate cases of OSF, the polarizing color of fibres is both red and yellowish orange as this stage contains both thick and thin collagen fibres. In advanced stage of OSMF, it was observed that the polarizing color of thick fibres showed red color. As the disease shows the severity, the color of the collagen fibres gets brighter. The intensity of brightness of collagen viewed with polarized light depends not only on collagen birefringence but also on the orientation of fibres with respect to the polars. However, the tight packing of collagen fibres in severe cases resulted in orange red- red color throughout the sections.

Sl.No	Histopathological grades	Number of cases	Percentages (%)
1	Grade 1 (mild)	12	26.1
2	Grade 2 (moderate)	14	30.4
3	Grade 3 (severe)	10	21.7
4	Normal (control group)	10	21.7
5	TOTAL	46	100.0

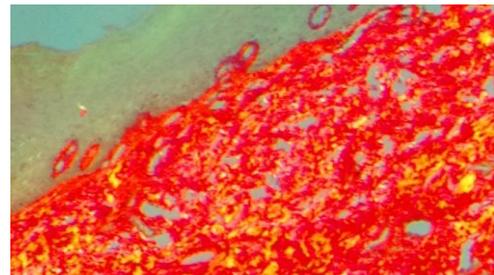
**Table 1: Total number of OSF cases with respect to the histopathological grading.**

Histological grading	Thin fibres (upper half)	Thin fibres (lower half)
	MEAN±SD µm	MEAN±SD µm
Grade 1	4.33±0.65 µm	4.00±0.74 µm
Grade 2	3.14±0.86 µm	2.86±0.77 µm
Grade 3	3.20±0.63 µm	2.50±0.85 µm
NORMAL	2.04±1.55 µm	1.72±1.68 µm
TOTAL	3.23±1.23 µm	2.83±1.30 µm

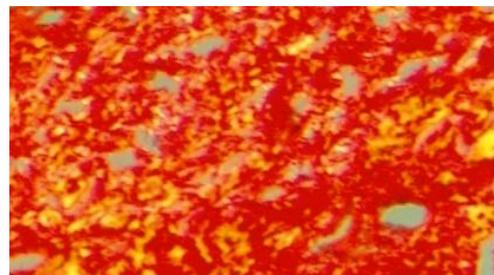
**Table 2: showing average number of thin fibers in the lower half and upper half of the connective tissue**



**Statistical Analysis: Paired t test. P value =0.006 Significant at 5% level of significance.**



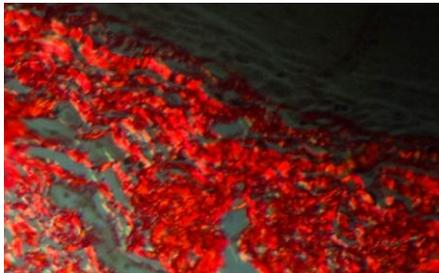
**Fig 1: Photomicrograph showing parallel orientation of collagen fibres in grade II OSF patients observed using polarizing microscope.**



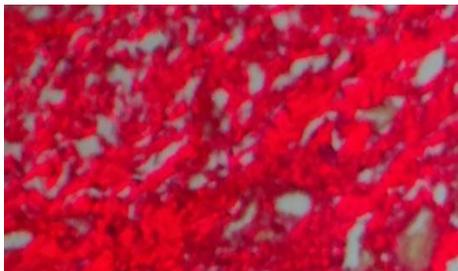
**Fig 2: Photomicrograph showing both thin and thick collagen fibres. Thin fibres showing yellow-orange colour and thick fibres showing orange-red colour.**

Histological grading	Thick fibres (upper half)	Thick fibres (lower half)
	MEAN±SD µm	MEAN±SD µm
Grade 1	5.33±0.89 µm	6.33±0.65 µm
Grade 2	7.00±0.68 µm	7.29±0.83 µm
Grade 3	12.40±1.58 µm	14.80±1.40 µm
NORMAL	8.97±2.14 µm	8.14±2.28 µm
TOTAL (GRADE 1,2,3 & Normal)	8.17±2.90 µm	8.86±3.49 µm

**Table 3: Average number of thick fibres in the lower half and upper half of connective tissue**



**Fig 3: Photomicrograph showing orientation of collagen fibres parallel to the epithelium in grade III OSF**



**Fig 4: photomicrograph showing thick bundles of collagen fibres in lower half of the connective tissue in orange-red color in grade III OSF**

#### IV. DISCUSSION

Oral Submucous Fibrosis (OSF) is a high risk precancerous condition characterized by changes in the connective tissue fibres of the lamina propria and deeper parts leading to stiffness of the oral mucosa and restricted mouth opening.<sup>11</sup>

Blanching in oral mucosa clinically may be due to few conditions like scleroderma, anaemia, leukoplakia and OSF, which has a characteristic clinical appearance from early to advanced stages in forms of palpable fibrous bands running vertically in the buccal mucosa.

A larger number of studies have been reported in the literature on changes in oral mucosa in OSF. Still, the question of fibrous bands running vertically and not horizontally has not been addressed when observed clinically.<sup>12</sup>

The present study is aimed histologically to ascertain the direction, quality organisation and packing of collagen fibres in different histopathological grading of OSF.

Our results are consistent with other studies which showed the parallel orientation of collagen fibres with respect to the

epithelium and the intense colour of the collagen fibres in different grades of OSF.<sup>5, 9, 12</sup>

The upper half of the connective tissue in grade I OSF cases showed parallel orientation of collagen fibres to the epithelium and more number of thin fibres that are yellow-orange in colour where as the lower half of the connective tissue showed mixture of both thin and thick fibres, in grade II OSF cases showed parallel orientation of collagen fibres to the epithelium and less number of thin collagen fibres compared to thick number of collagen fibres where as the lower half showed thick collagen fibres in orange red in colour as shown in fig.1 and fig.2.

In grade III OSF cases, the upper half and lower half of the connective tissue revealed thick and red- orange collagen bundles that are tightly packed as shown in fig.3.

The difference in colour patterns of collagen fibres could be due to various growth factors and cytokines that cause proliferation of fibroblasts and extracellular matrix resulting in the formation of thick mature collagen. As collagen matures, the change in the proteoglycan content of fibre causes dehydration of the fibres thereby increase in diameter of collagen fibres. The colour red-orange may be due to the tight packing of the collagen fibres. Hence, there is increase in intensity of birefringence and change in the polarizing colours.<sup>13</sup>

The cause for these unidirectional or parallel alignments of collagen fibres may be due to:

1. Chronic stimulation of oral mucosa by irritation or as sequence of mechanical stress where the fibres orient with the direction exerted on them like in the scars, keloid.
2. Due to forces generated by cell-mediated gel contraction.
3. Due to changes in the extracellular matrix imbalance production and degradation.

#### V. CONCLUSION

Picrosirius stain is most suitable to visualize collagen fibres quantitatively by determining their length in plane of tissue section and for contrast of stained fibres against background which enables qualitative analysis. The application of stain is a relatively simple tool to study the changes in extracellular matrix in particular the structural integrity of collagen fibres induced by stress in diff grades of OSF which in turn causes the neoplastic change in epithelium.

#### VI. DRAWBACKS AND LIMITATIONS

Though picrosirius red stains very thin collagen fibres in comparison to other collagen stains. Factors like pH, concentration of stain and the duration of staining will attribute to the variation in results. It is not advisable to use the staining technique on tissue preserved in formalin for many number of days. Hence, researchers must aim at ultra structural features of connective tissue in different stages of OSF in future.

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