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## Original Article

# Polarized microscope assisted study of collagen fibres in Oral Submucous Fibrosis and their clinicopathologic correlation: An in-vitro study

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## ABSTRACT

**Background:** The present study was aimed to evaluate and analyze orientation and birefringence pattern of collagen fibers histopathologically in different grades of OSMF using the special stain (Picrosirius red) under polarizing microscope and relate these factors with the severity of OSMF.

**Materials and Method:** 45 confirmed cases of OSMF were taken from the archives. The selection of clinical cases was done according to classification by J.N Khanna and Andrade (1999). These cases were correlated histopathologically as per the criteria given by Pindborg J.J and Sirsat S.M (1966). The sections were stained with H & E and Picrosirius red stains and assessment was done accordingly. 10 cases of normal mucosa were taken as control.

**Results:** In clinical grade I, 60% of the cases showed histopathological grade II, while 53.4% cases in clinical grade II correlated with histopathological grade III and 33.4% of clinical grade III cases showed histopathological grade IV. Upon application of Fisher's exact test a significant P value <.01 was obtained suggesting no statistical significant correlation between these two parameters. However, the orientation pattern revealed a parallel orientation as OSMF advanced. Also as the severity of the disease increased, there was a gradual shift from green- greenish yellow colour of the fibres and a shift to orange red-red colour.

**Conclusion:** Upon correlation of clinical grades with histopathological grades, no statistically significant difference was found. Birefringence pattern of collagen revealed similar findings as other studies in case of clinical grades but histopathological grades showed a contrast finding.

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## 1. Introduction

The first ever mention of Oral Submucous Fibrosis (OSMF) was by SUSHRUTA in his classification of mouth and throat disorders in which he called it 'VIDARI'. He described features of progressive narrowing of mouth, depigmentation of oral cavity and pain on eating food associated with the condition.<sup>1</sup> OSMF has also been described as 'Atrophica idiopathica tropica mucosae oris' (Schwartz, 1952). Joshi (1953) coined the term 'Submucous fibrosis of the palate and pillars'. Other names that have been suggested are 'Diffuse oral submucous fibrosis' (Lal L, 1953), 'Idiopathic

scleroderma of the mouth' (Su J.P. 1954), 'Idiopathic palatal fibrosis' (Rao A.R.N, 1962) and 'Sclerosing stomatitis' (Behl P.N, 1962).<sup>1</sup>

OSMF is defined by Pindborg<sup>1</sup> as "an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by fibro elastic change of the lamina propria, with the stiffness of the oral mucosa, trismus and inability to eat"<sup>1</sup>.

The disease mainly occurs in Indian subcontinent and affects 0.2-1.2% of the urban population. It has a female preponderance and mostly presents in the middle life, mean age being 43 years. Initial signs & symptoms of the disease are inability to tolerate spicy food and blanching of the oral mucosa usually symmetrically.

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As the disease progresses, thick inelastic fibrous bands appear in the sub mucosal layer of the mucosa, faucial pillars, floor of mouth and tongue. In advanced cases, uvula becomes atrophic, the mobility of soft palate and tongue is markedly reduced and there may be atrophy of tongue papillae. In most advanced cases, it can lead to difficulty in mastication, deglutition and phonation. It is also associated with epithelial dysplasia and there is an increased risk of carcinomatous changes, hence it is considered to be a precancerous condition.<sup>2</sup> Malignant transformation of OSMF has been reported to be around 7.6%.<sup>2</sup>

OSMF is believed to have a multifactorial etiology. A number of factors that trigger the disease process are mainly areca nut (Betel nut) chewing, ingestion of chilies, nutritional deficiencies and genetic alterations. Constant immunological abnormalities in patients with OSMF are found which could have implication for the malignant transformation of the lesions.<sup>3</sup>

Betel quid (BQ) is the fourth most common substance of misuse in the world after tobacco, alcohol and caffeine. The areca nut (Betel nut) is a component of Betel quid (BQ). The flavonoid content of areca nut has direct influence on collagen metabolism causing OSMF. The Areca nut is the seed of the areca palm (Areca catechu). It is not a true nut but rather a drupe and is commercially available in dried, cured and fresh forms.<sup>4</sup>

Areca nut is a mild stimulant, causing a hot sensation in the body, heightened alertness and sweating, although the effects vary from person to person. The areca nut contains tannin, Gallic acid, a fixed oil gum, a little terpineol, lignin, various saline substances and three main alkaloids: Arecoline, Arecaidine, Guacine which have vasoconstricting properties.<sup>4</sup> Areca nuts also have been shown to have a high copper content, and chewing areca nuts for 5–30 minutes significantly increases soluble copper content in oral fluids which might account for fibrosis in OSMF.<sup>5</sup>

The quantitative determination of collagen is time consuming and not very sensitive. To overcome this problem, Picrosirius red stain may be used as an effective stain in assessment of collagen fibres in OSMF. Sirius red is a strong cationic dye which stains collagen by reacting via sulfonic acid groups with basic groups present in the collagen molecules. The elongated dye molecules are attached to collagen fibers in such a way that their long axis is parallel. This parallel relationship between dye and molecules results in enhanced birefringence. The role of picric acid is to prevent the indiscriminate staining of non-collagenous structures by Sirius red.<sup>6,7,8</sup>

This study was therefore, designed to evaluate and analyze the orientation and birefringence pattern of collagen fibers histopathologically in different grades of OSMF using Picrosirius red stain under polarizing microscope and to relate these factors with the severity and progression of OSMF.

## 2. Materials and method

The present study was carried out on formalin fixed, paraffin embedded tissues of histopathologically diagnosed cases of Oral submucous fibrosis retrieved from the archives of the Department of Oral Pathology. Clinical details of the patients were obtained from the records of Department of Oral Medicine and Radiology. Cases were clinically graded as per the criteria given by Khanna and Andrade.<sup>9</sup> Histopathological grading of OSMF cases was done as per the criteria given by Sirsat and Pindborg.<sup>10</sup>

Of the selected 45 cases, 15 cases each were selected in three clinical grades. For each case, 2 sections were made, of which one section was stained with routine H&E stain, used to grade the cases histologically, while the other section was stained with special stain Picrosirius red to assess the collagen fibers orientation and birefringence pattern, thus analyzing the maturation of collagen. 10 cases of normal buccal mucosa were selected as controls for

comparison. For H & E and Picrosirius stains the methods were followed as suggested by Suvarna et al.<sup>11</sup>

The Picrosirius red stained sections were viewed using polarized microscopy. In bright field the collagen fibres appeared red on a pale yellow background while nuclei were gray to brown in color. However, when examined through crossed polars, the largest collagen fibers were bright yellow to orange, and the thinner ones yellowish green to green. Upon application of Fisher's exact test a significant p value <.01 was obtained.

## 3. Results

Upon correlation of clinical grades with histopathological grades, it was noted that out of 15 clinical grade I cases, 60% of the cases showed histopathological grade II, similarly in clinical grade II 53.4% of the cases were in histopathological grade III. In clinical grade III, 53.4% of cases were in histopathological grade III and 33.4% were in histopathological grade IV (Fig. 1).

A comparison of the orientation of collagen fibers in each clinical grade of OSMF was done and it was found that out of total cases in clinical grade I, 13.4% cases showed parallel orientation, 40% cases showed non-parallel orientation, and a mixed orientation was observed in 46.7% cases. In clinical grade II, 53.4% cases showed parallel orientation, 26.7% revealed a non-parallel orientation and mixed orientation was noted in 20% cases. In clinical grade III, parallel orientation was noted in 33.4% cases, non-parallel orientation was noted in 26.7% cases and mixed orientation was noted in 40% cases (Fig. 2).

However an overall comparison of orientation of collagen fibers in different histopathological grades was done and the results have been tabulated in Table 1. As regards to the birefringence pattern of collagen fibers in each clinical grade of OSMF a comparison was done. It was noted that out of 15 cases in clinical grade I, 33.4% cases showed yellow orange fibers. In clinical grade II, 13.4% cases showed yellow orange fibers and 13.4% cases showed red fibers. In clinical grade III, 26.7% cases showed red fibers (Fig. 3).

Similarly an analysis of birefringence pattern in each histopathological grades was done and it was noted that out of 12 cases in histopathological grade II, 41.7% cases showed yellow orange fibers; whereas in histopathological grade III, out of 21 cases, 23.8% cases showed yellow orange fibers; and in histopathological grade IV, out of 12 cases, 16.7% cases showed (Fig. 4). The varying birefringence pattern of collagen fibres in oral submucous fibrosis has been depicted in Fig. 5.

## 4. Discussion

Prevalence of OSMF has been on the rise, which at present is attributed to the use of arecanut and its products in variety of ways. Lack of awareness, aggressive marketing strategies of these products and failure to impart primary preventive steps have also contributed in the spread of the disease in a considerable manner among the rural and urban younger population. This is evident from the fact that the number of OSMF patients approaching for medical care in very early stages of the disease is probably a miniscule of total number of patients affected by the disease. The gravity of the problem can be assessed by the fact that by 1998, total estimated number of patients in India affected by the disease was approximately 2 million cases,<sup>12</sup> which is an eight fold increase in number, when compared to the reports by J.J Pindborg.<sup>13</sup> Studies on collagen in OSMF has been a major focus in research as arecoline, the main alkaloid constituent of the arecanut has been reported to induce excessive collagen synthesis.<sup>7</sup> Studies have shown that arecoline induces collagen formation, demonstrates cytotoxicity and also stimulates double stranded nucleic acid synthesis and cell- morphology change.<sup>9,14</sup>

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