International Journal of Multidisciplinary Research and Modern Education (IJMRME)
Impact Factor: 6.725, ISSN (Online): 2454 - 6119
(www.rdmodernresearch.org) Volume 3, Issue 2, 2017

HISTOPATHOLOGICAL ANALYSIS OF COLLAGEN WITH PICROSIRIUS RED STAIN IN ORAL SUBMUCOUS FIBROSIS UNDER POLARISING MICROSCOPE

S. Swetha* & Dr. S. Gheena**
* BDS (Third Year), Saveetha Dental College and Hospitals, Chennai, Tamilnadu
** Assistant Professor, Department of Oral Pathology, Saveetha Dental College and Hospitals, Chennai, Tamilnadu

Cite This Article: S. Swetha & Dr. S. Gheena, "Histopathological Analysis of Collagen with Picrosirius Red Stain in Oral Submucous Fibrosis Under Polarising Microscope",International Journal of Multidisciplinary Research and Modern Education, Volume 3, Issue 2, Page Number 17-21, 2017.

Abstract:
BackGround: Oral Submucous Fibrosis (OSMF) is potentially malignant condition of the oral cavity in which there is excessive deposition of collagen in connective tissue resulting in trismus and inability to eat. It is caused predominantly by areca nut chewing. It shows characteristic histopathological features like increased collagen fiber formation in the initial stages followed by dense collagen fiber bundles and different degrees of hyalinization, along with atrophic epithelium.
Aim: To evaluate the changes in birefringence of collagen fibres stained with picrosirius red stain and compare with hematoxylin and eosin stained sections.
Materials and Methods: The study included 5 diagnosed cases of OSMF in which 4 cases were advanced stages of OSMF and 1 case was moderately advanced stage of OSMF. Two sections from each tissue block were prepared. One of the sections were stained with Hematoxylin and Eosin (H&E) and other with picrosirius red. The H&E stained slides were examined under light microscope and picrosirius red stained slides were examined under polarising microscope. Results were then analysed statistically.
Results: On evaluation of polarising colours and intensities of birefringence of collagen fibres in lamina propria, collagen deposition around the muscle and collagen deposition around the blood vessels shows pale yellow birefringence predominantly. This reveals the collagen fibres are thick and are more tightly packed.
Conclusion: The results of present study show a significantchange in birefringence and arrangement of collagen fibres between the variouscomponents of connective tissue. This gives anassumption of impending neoplastic change in OSMF.
Key Words: Collagen Fibres, Oral Submucousfibrosis, Picrosirius Red Stain& Polarising Colours

Introduction:

Oral Submucous Fibrosis (OSMF) is a chronic disease of insidious onset and a prevailing potentially malignant disorder characterized by juxta-epithelial inflammatory reaction along with mucosal fibrosis in lamina propria and atrophy of epithelium causing trismus and inability to eat .It is a well recognised entity with frequency ofmalignant transformation reported in the range of 7-13%(1,2).The condition is usually preceded by symptoms like burning sensation of oral mucosa,ulceration and pain. The characteristic features that follow the initial symptoms include restricted movement and depapillation of the tongue, blanching and leathery texture of oral mucosa and progressive reduction of mouth opening(3).

Collagen forms a major portion of connective tissue and provides it with a unique combination of flexibility and tensile strength, but is not very elastic. The structural integrity and tissue function is maintained by collagen (4).Increase in the rate of collagen synthesis and decrease in collagen degradation play a key role in the pathogenesis of OSMF. OSMF shows characteristic histopathological features like increased collagen fiber formation in the initial stages followed by dense collagen fiber bundles and different degrees of hyalinization, along with atrophic epithelium.

Traditional stains such as VanGieson and trichrome were used to detect collagen fibres,however these stains failed to demonstrate thin fibres . The connective tissue integrity is maintained by both thick and thin fibres, emphasizing the importance to demonstrate them in order to evaluate any connective tissue changes(5). Picrosirius red a special stain was found to be promising to demonstrate both thin and thick fibres, which stains the thin fibres intensely and increases their birefringence.

The study was conducted to evaluate the changes in birefringence of collagen fibres stained with picrosirius red stain and compare with hematoxylin and eosin stained sections.

Materials and Method:

The study included 5 diagnosed cases of OSMF in which 4 cases were advanced stages of OSMF and 1 case was moderately advanced stage of OSMF. Two sections from each tissue block were prepared. One of the sections was stained with Hematoxylin and Eosin (H&E) and other with picrosirius red.

Picrosirius Red Stain for Collagen:

5-micron thick sections were floated on to micro slides and incubated for 1 hour at 480C on a slide warmer for proper adhesion of the section on to the slide. Sections were then deparaffinized in xylene and hydrated through decreasing grades of alcohol. These sections were incubated in 0.1 % (W/V) Sirius red F3B (BX 10934, Bayer chemicals) in saturated Picric acid solution for 1 hour at roomtemperature. This was followed by rinsing with distilled water, staining with Mayer's haematoxylin, differentiation in 1% Hcl, alkalinization with tap water, dehydration andmounting. The H&E stained slides were examined under light microscope and picrosirius red stained slides were examined under polarising microscope.

Evaluation:

The H&E stained slides were observed to record the following parameters:

- ✓ Presence or absence of hyalinization in the lamina propria
- ✓ Dynamic state of the blood vessels (dilated/constricted)
- ✓ Density of connective tissue (loose fibrillar/ densebundular / moderately dense bundular)
- ✓ Deposition of collagen around muscles
- ✓ Deposition of collagen around blood vessels

The picrosiriusred stained slides werereviewed for different polarizingcolors of collagen in lamina propria, around muscle and around blood vessel. Various groups of polarizingcolors were observed which included red, intense red, greyish red, orange red, moderate red and pale yellow. Results were then statistically analyzed.

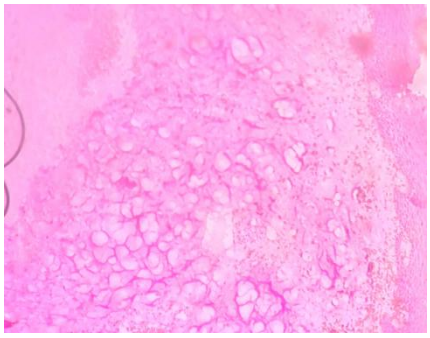


Figure 1: Juxtaepithelial Hyalinisation

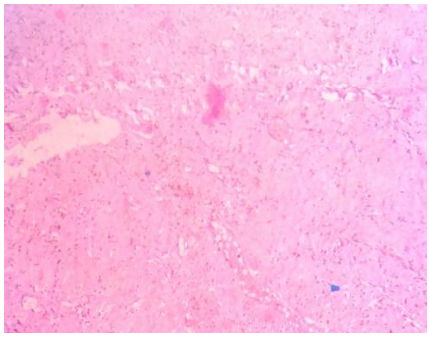


Figure 2: Dense Collagen Fibres around Blood Vessels

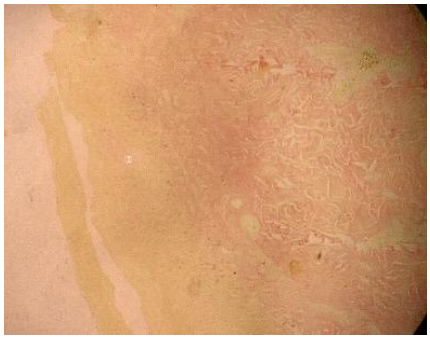


Figure 3: Collagen Fibres Showing Birefringence under PolarisingMicroscope

Results:

On evaluation of haematoxylin and eosin stained sections under light microscope, hyalinisation in lamina propria was found to be 60%, density of connective tissue were predominantly moderate dense bundle like (60%), blood vessels in lamina propria were dilated (100%), collagen deposition around the muscle were sparse (40%) and collagen deposition around the blood vessels were dense(100%) (Table 1, Fig 4). On evaluation of picrosirius red stained sections for polarising colours and intensities of birefringence of collagen

fibres in lamina propria, collagen deposition around the muscle and collagen deposition around the blood vessels shows pale yellow birefringence predominantly (40%) . This reveals the collagen fibres are thick and are more tightly packed. (Table 2, Fig 5)

Table 1

PARAMETERS		%
Hyalinisation in lamina propria	Present	60%
	Absent	40%
Density of connective tissue	Loose fibril like	20%
	Moderately dense bundle like	60%
	Dense bundle like	40%
Blood vessels in lamina propria	Constricted	0%
	Dilated	100%
Collagen deposition around muscle	Dense	20%
	Sparse	40%
Collagen deposition around blood vessel	Dense	100%
	Sparse	0%

Figure 4

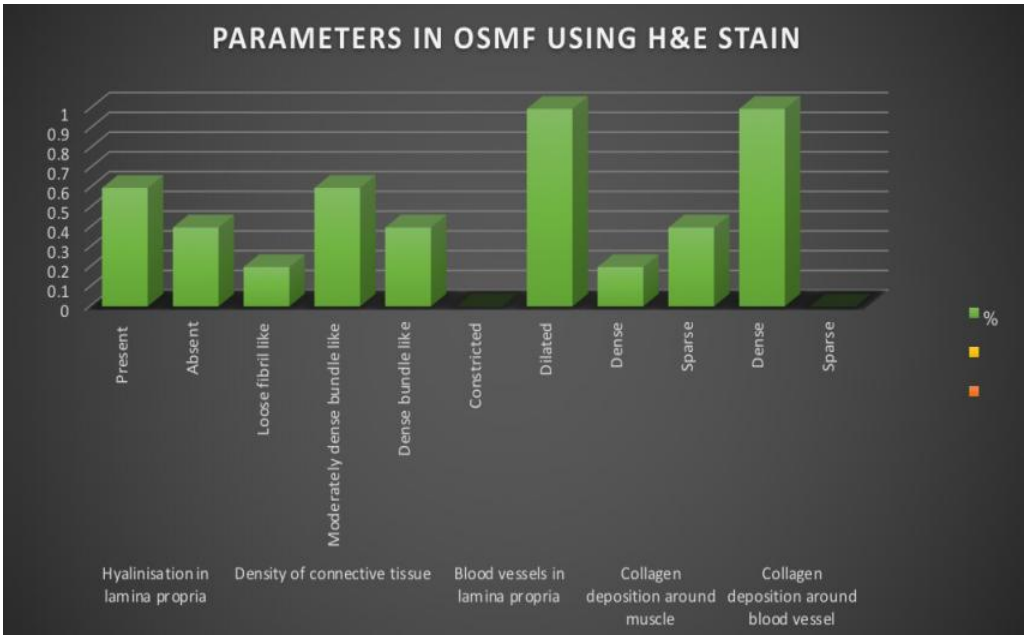
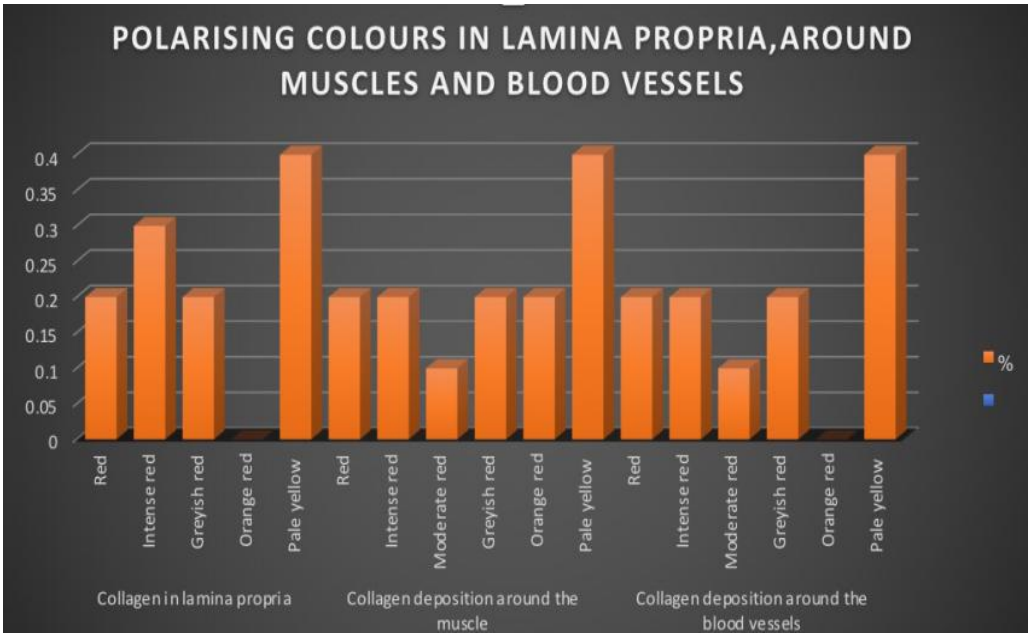


Table 2

PARAMETERS	POLARISING COLOURS	%
Collagen in lamina propria	Red	20%
	Intense red	30%
	Greyish red	20%
	Orange red	0%
	Pale yellow	40%
Collagen deposition around the muscle	Red	20%
	Intense red	20%
	Moderate red	10%
	Greyish red	20%
	Orange red	20%
	Pale yellow	40%
Collagen deposition around the blood vessels	Red	20%
	Intense red	20%
	Moderate red	10%
	Greyish red	20%
	Orange red	0%
	Pale yellow	40%

Figure 5



Discussion:

Abnormal collagen deposition in the connective tissue of OSMF is directly related to arecanut (betel nut), a component of the betel quid, which induces an increase in the turnover of collagen(6,7). Fibroblasts are stimulated by the alkaloids and the flavanoids present in the areca nut resulting in the accumulation of collagen . The collagen thus accumulated , exhibits increased cross linking , along with reduced collagenase activity results in decreased collagen degradation(8). Currently many researchers have observed that Sirius red stain enhances the normal birefringence of collagen fibers in tissue section (9). Thin collagen fibers exhibit green to greenish yellow polarizingcolors, whereas thick fibers reveal yellowish-orange through orange to red polarizingcolors with picrosirius red stain. Green to greenish-yellow colours implicates poorly packed collagen whereas orange red color denotes tightly packed fibers (10, 11). The color and intensity of birefringence are due to the difference in their pattern of physical aggregation and thickness of collagen fibres .

In the present study , the color intensities of birefringence in control sections were predominantly pale yellow towards basement membrane, around muscle and around blood vessel. Thus the collagen fibres were thick and tightly packed . While the deeper lamina propria showed predominantly yellowish -orange to reddish - orange birefringence as observed by Montes et aland Junqueira et alin their respective studies.

The density of collagen fibersincreased as the disease progressed . This is because the fibroblastic activity is modulated by interaction of these cells with local factors present in the oral mucosa . In addition to the inflammatory cells , arecolinethe main alkaloid in betel nut extracts could stimulate fibroblastic proliferation and collagen synthesis(12,13,14). It was also found that the activity of lysyl oxidase is increased in OSMF fibroblasts compared to normal fibroblasts (15, 16).Although considerable amount of work has been done on collagen , not many techniques have been proposed to demonstrate this protein adequately in tissue sections . Picrosiriusred stain was proved to be excellent in demonstrating both thick and thin collagen fibres by enhancing their birefringence.

Conclusion:

Oral submucous fibrosis being the most commonpotentially malignant disorder, with changes in thestructural integrity of collagen leading to the dysplasticchange in the epithelium resulting in carcinoma.Picrosirius red staining is a most simple technique tovisualize changes in the collagen both quantitatively andqualitatively.

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