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The assessment of role of collagen fiber in oral submucous fibrosis, oral squamous cell carcinoma and oral submucous fibrosis with oral squamous cell carcinoma by using Picro Sirius red staining & polarized microscope.

Madhuri Gawande [1], Ashwini Nareshchandra Walke [2], Swati Patil[3], Meenal Choudhary[4]

[1](*Professor & Head of department of Oral Pathology and microbiology, S. P. Dental College & Hospital, Sawangi (M), Wardha, Maharashtra, India)*
[2](*PG student, 3rd year, Department of Oral Pathology, S. P. Dental College & Hospital, Sawangi (M), Wardha, Maharashtra, India)*
[3](*Professor, Department of Oral Pathology and microbiology, S. P. Dental College & Hospital, Sawangi (M), Wardha, Maharashtra, India)*
[4](*Professor & Vice Dean (Academic) S. P. Dental College & Hospital, Sawangi (M), Wardha, Maharashtra, India)*

Abstract : *About 0.5% of Indian population is affected by oral submucous fibrosis (OSMF) and it has generated considerable interest in medical science field because it is posing a major health problem. Along with OSMF, oral squamous cell carcinoma (OSCC) comprises a bulk of all oral malignancies, and it has been proven by studying that OSMF has 7-13% malignant potential. Hence, the study was carried out to study collagen fiber histopathologically for the orientation and thickness of collagen fibers in OSMF, OSCC and OSCC with OSMF with picrosirius red staining and was examined with polarizing microscope.*

Material & method: *Each fifteen cases of histologically diagnosed, OSMF, OSCC and OSCC with OSMF were taken for the study and fivespecimen of normal buccal mucosa were also incorporated as a control group. All were stained with haematoxylin, eosin and picrosirius red staining for evaluation under polarised microscope.*

Result: *In the study, we found there is a gradual change in birefringence of collagen fibers from red to orange red to yellowish green as from OSMF to OSMF with OSCC and to OSCC.*

Conclusion: *The present study indicated that the change in orientation and thickness of collagen fibers in OSMF could acts as a prognostic marker indicative of malignant transformation.*

Keywords: *Malignancies, Picro Sirius red, polarized microscope*

I. Introduction

OSMF is not only a well-recognized malignant condition of oral cavity illustrated by inflammation as well as progressive fibrosis of lamina propria and deeper connective tissue[1,2] but also most frequently encounter premalignancy and a never ending illness with dangerous onset, affecting the pharynx and upper digestive system as well. In 1952, OSMF was acknowledge condition as "Atrophiaidiopathica (tropica) mucosae oris"in five Indian women in Kenya.[1,2] About 0.5% of Indian population is affected by OSMF.[3] Since then, the disease has generated huge interest in the field of medical science. Epithelial precancerous development that leads to well documented molecular and structural changes in the epithelium. Recently, it has been recognized that stromal biology is also altered significantly with preinvasive disease.[4] OSCC comprises a bulk of all oral malignanciesand it has been proven by studied that OSMF has 7-13% malignant potential.[1]

In OSCC, cell invade the stroma in the form of islands, strands or sheets, which are embedded or surrounded by an extracellular matrix, thus producing reactive changes in the stroma.[5] There is also concentrate deposition of collagen fibers in the connective tissue stroma due to decrease in its degradation by various inhibitory mechanisms.[6]The synthesis of collagen is mediated by various growth factors, hormones, cytokines, lymphokines and TNF-β.[6] This collagen in extracellular matrix having important role in maintaining structural integrity and tissue functions of the body and is consider as a barrier for the spread of the tumor also. The proteolytic remodeling of extracellular matrix leads to abundant changes in the collagenous stroma, helps in tumor invasion and progression.[4]

As collagen is a basic amino acid and has a strong affinity for acidic dyes. Sirus red is an elongated dye molecule responds with the collagen and increases the birefringence property of collagen.[1] Many methods to detect, quantify and evaluate the nature of collagen are available, in a few studies, it was found they were using

the picroserous red stain as it has capability to detect thin collagen fibers which are not possible with routine staining procedure and helps to differentiate between mature and immature collagen fibers.[4]

In this study, collagen was studied histopathologically for the orientation and thickness of collagen fibres in OSMF, OSCCand OSCCwith OSMFwith picrosirius red staining and examined with polarising microscope. The present study helps in documentation of connective tissue changes in OSMF, OSCC and OSCCwith OSMF. The objectives set was tostudy the orientation and thickness of collagen fibres in OSMF, OSCCand OSCCwith OSMF.

II. Material & Method

Fifteen paraffin embedded blocks of histopathologically diagnosed cases each of OSMF, OSCC and OSCC with OSMF were obtained from the archives of the department of Oral Pathology and Microbiology and 5 cases of normal buccal mucosa were included in the study. The OSCC & OSMF & OSMF with OSCC sections were stained subsequently with picrosirius red (Sirius red in a saturated picric acid solution,[7,8] which were evaluated under polarising microscope. Only Stage IIIOSMF (moderately advanced) were considered in this study grading for which done according to Pinbrg et.al histopathological grading system for OSMF.[8] For OSCC, grading was done according to Brynes histological malignancy grading system,[9]and moderately differentiated OSCC cases were considered and cases of OSMF which were transformed to OSCC were included in the study.

The sections were observed under polarizing microscope. A total of 10 high power field from each section are visualized and their color was noted for interpretation and comparison of collagen fiber hue as show in table.1.

III. Results

The study included total 50 cases in which 5 were the control group, which shows haphazardly arrangned collagen fibers in the connective tissue stroma with respect to epithelium. Ten cases of OSMF showing predominently thick collagen fibers which are having orange red to reddish birefrengence. (Figure 1) The reason for this finding is the collagen fibers in OSMF is hyalinized and tightly packed, also the less number of thin fibers giving rise to red colour through out the section. And 12 cases shows the collagen fibers in OSMF were oriented parallel to the epithelium(Figure 2).

In OSCC, 10 cases were showing predominantely greenish yellow birefringence (Figure 4). The reason for this is thought to be the decrease in thick fibers and more accumulation of thin fibers giving greenish yellow color to the section.Also 13 cases showing the collagen fibers are arranged haphazardly in relation with epithelium (Figure 3).

In OSMF with OSCC we observed 8 cases giving predominantly greenish yellow birefriengence and 4 case showing yellow orange birefrengence (Figure 5) and 4 cases giving a mixed picture of these two birefriengence. Also in OSMF with OSCC, 6 cases showing predominently parallel collagen fibers in relation with the epithelium. 7 cases was showing haphazardly arrenged collagen fibers and 2 cases were showing combination of both (Table 2).

In this study we found that, there is gradual change in birefriengence of collagen fibers from red to orange red- yellowish orange to yellowish green as from OSMF to OSMF with OSCC and to OSCC.

IV. Figures and table

Figure 1: Photomicrograph (10X) shows OSMF showing reddish birefrengence.

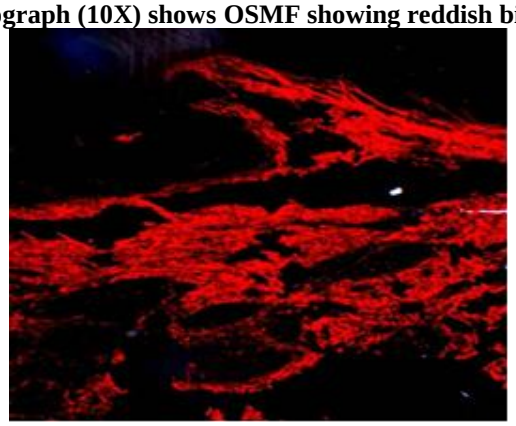

Figure 2: Photomicrograph (10X) shows OSMF showing reddish birefrengence with collagen fibers arranged parallel in relation to epithelium.

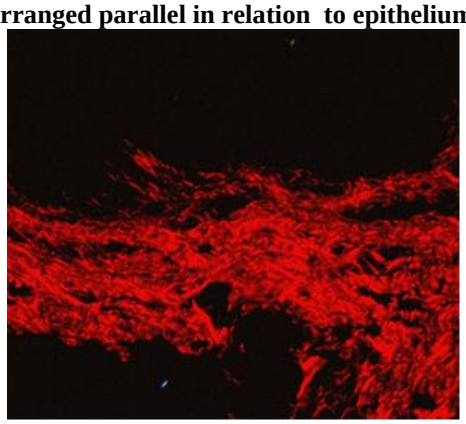

Figure 3:Photomicrograph (10) of OSCC showing haphazardly arrangment of collagen fibers in relation to epithelium.

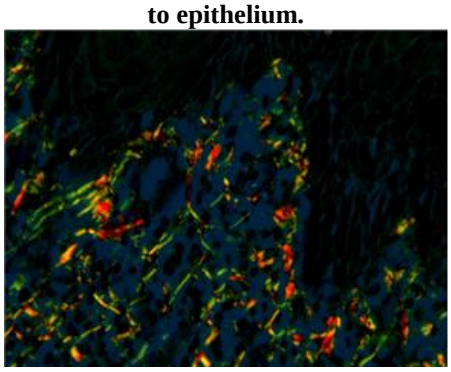

Figure 4: Photomicrograph of OSCCshowing predominantly giving greenish yellow birefriengence.

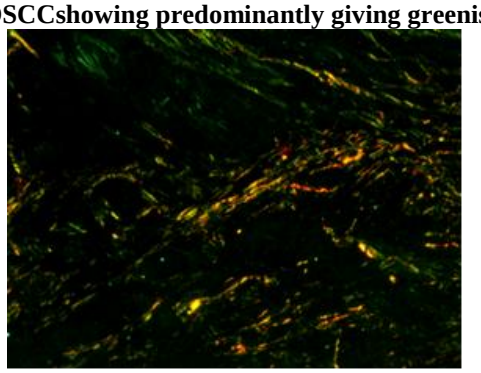

Figure 5: Photomicrograph 10X shows OSMF with OSCC showing predominently yellow orange birefriengence

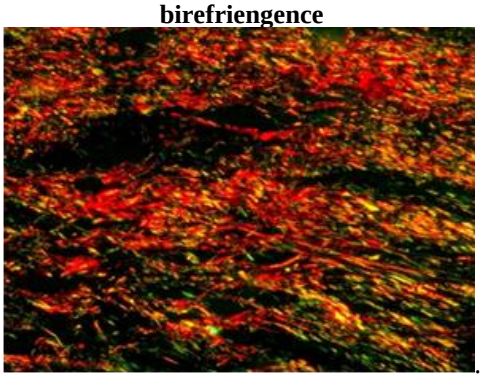

Table 1. Assessment &differences in the polarizing colors based on hue.

Thick fibers	Dark red, reddish orange.
Thin fibers	Greenish yellow.
Combination of both thik and thin fibers	Yellow orange to greenish yellow.

Table 2. Assessment &Differences in orientation of collagen fibers in relation to epithelium with polarizing colors based on hue.

Connective tissue Changes with	Total no. of cases	Predominantly parallel to the epithelium		Predominantly haphazardly arranged		Combination of both	
		No.	%	No.	%	No.	%
OSMF	15	12	80%	3	20%	0	0%
OSCC	15	0	0%	13	86%	2	14%
OSCC + OSMF	15	6	40%	7	46%	2	14%

Table 3.Assessment &Differences in thickness of collagen fibers with polarizing colors based on hue.

Study group	Total no. Of Cases	Predominantly thick Fibers		Combination of thick and Thin fibers		Predominantly thin Fibers	
		No.	%	No.	%	No.	% %
OSMF	15	10	67%	3	20%	2	13%
OSCC WITH OSMF	15	3	20%	4	27%	8	53%
OSCC	15	1	6%	4	27%	10	67%

V. Discussion

As stated in the leterature that, elements which plays important role in tumour progression are stromal elements which surrounds the tumour, that includes endothelial cell, fibroblasts and inflamatory cell[10] as a know fact collagen is triple helical structutre and its molecules are affluent in basic amino acid, therefore, having strong affinity for acidic dyes giving strong reaction with the acidic dyes. Picrosirius red is acidic dye that is having six sulfonic group, so used to stain collagen in tissue sections.[4,11]

The orange red- red colour of collagen which was obsreved in cases of OSMF was because of tight packing of collagen fibers suggestive of presence of thick fibers in the extacellular matrix.[12,13]As the collagen in OSMF grade III cases were tightly packed bundles, so it difficult to identify individual collagen fibers, this is due to increseed in thickness of collagen fibers as the disease advanced.[14]

The colour red is owing to firm wrapping of collagen fibers that's why there is decreased intensity of birefrengence and alteration in polarization colour due to which changes in extracellular matrix, imbalance between production and degredation.The gradual change from yellowish orange to greenish yellow was more significant when OSMF is transforming to OSCC.[15]The haphazardly arrangement of collagen fibers was indicative of incresed collagenolytic enzyme activity which were obseved during transformation of preneoplastic to carcinoma stage.[15]Also when premalignant state is trnasformed to carcinoma there is incresead in collagenolytic enzyme activity.[15]Tumor cell produces collagenases which has ability to degrade Type-I collagen and helps in invasion and metastsis.[16]

Many authers suggested that the subunits of collagen become thinner to form a fibers. Also many of them suggested that the periodicity of collagen interlinking is not changed and the collagen form is the normal.[17]There are two origin of collagen fibers to be sugested , one is tumor cell origin which helps the tumor cell to progress reducing in the thickness.Another is the stromal origin which prevent tumor spread further.[5]The origin of these fibers was considered to be the tumor cell fibroblast and the collagen by these cell is thought to be procollagen which is a pathologic collagen.[12,13]

In the present study, the cause of incresed deposition of collagen in the connective tissue stroma is due to decresed in collagenase activity.[18]The reson for this was, beetal quide which is the primary cause of OSMF content areca nut, flavinoids, alkaloids which stimulates the fibroblast, causing increased deposition of collagen and also leads to stabilization of collagen structure.[19,20] Lots of work were done on collagen by various method we prefered picroserious red stain as it has capicity to demonstrate thin collagen with the help of polarized microscpy. As there was formation of dye and collagen parallel relationship which helps to enhance birefrengence.[14] The results of this study are consistance with other studies which illustrated the parallel orientation of collagen fibers with respect to epithelium and colour changes observed in OSMF, OSCC with OSMF and in OSCC.The disparity in colour pattern of collagen fibers might exist due to assorted growth factors and cytokines causing fibroblast proliferation and extracellular matrix results in the arrangement of immature collagen.[6]

Since the collagen is converted from mature to become immature, the modification in proteoglycan content of fiber would be reason of dehydration of fibers thereby decressing the diameter of collagen fibers.

Type I collagen fiber showed a strong bireferengence of red , orange and yellow colour and a weak birefregence when the fiber were type III collagen so called thin fiber by polarized microscopy.[6,21]

According to Junqueira etal and Montes etal, the colour change can be atrributed to carcinogenic action of

MMPs. Pathological breakdown of matrix by tumor cells, promote tumor progression.[4]In the presentstudy, colour changes seen are clear indicator of stromal tissue alteration which can be related to carcinogenic events taking place during tumerogenesis.

Breast cancer studieds showsed that increased in collagen in extracellular matrix incresaes the mechanical stiffness and imparts resistance to the tumor.[22]Nuclear resonance studies by Sharf etal shows a colour orange correspondes with tight packing and greenish yellow to poorly packed fibers.[5]

This study further supported by, in follicular tyroid carcinoma higher frequency of yellow green collagen fibers at the site of invasion and orange red collagen fibers in noninvasive site.[23]Hence ,there is a corelation of change in polarization color of collagen fibers with the changes in the connective tissue stroma, which indicate connective tissue changes could also be indicative of neoplastic transformation.[15]

VI. Conclusion

Hence, the change in polarization colors from OSMF to OSCC is a radical change in the collagen orientation as well as thickness which could be indicative of numerous enzymatic actions, which are taking place as a part of change in connective tissue which occurred during malignant transformation. The observations of the present study indicated that the change in orientation and thickness of collagen fibers in OSMF could acts as a prognostic marker indicative of malignant transformation.

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References

[1]. Parveen S, Ahmed SA, Tanveer S. A Study on Orientation of Collagen Fibers in Oral Submucous Fibrosis. Group. 2013;10:21–7.
[2]. Schwartz J. Atrophia idiopathica (tropica) mucosae oris. 1952.
[3]. Smitha B, Donoghue M. Clinical and histopathological evaluation of collagen fiber orientation in patients with oral submucous fibrosis. J Oral Maxillofac Pathol JOMFP. 2011;15(2):154.
[4]. Kalele K, Managoli N, Roopa N, Kulkarni M, Bagul N, Kheur S. Assessment of collagen fiber nature, spatial distribution, hue and its correlation with invasion and metastasis in oral squamous cell carcinoma and surgical margins using Picro Sirius red and polarized microscope. J Dent Res Rev. 2014;1(1):14.
[5]. Aparna V, Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain-a histochemical study. J Clin Diagn Res. 2010;4:3444–9.
[6]. Ashalata G, Baghirath P, Krishna A, Kumar P, Tom A. Quantitative and qualitative analysis of collagen in oral submucous fibrosis. J Dr NTR Univ Health Sci. 2012;1(2):99.
[7]. Montes G, Junqueira L. The use of the Picrosirius-polarization method for the study of the biopathology of collagen. Mem Inst Oswaldo Cruz. 1991;86:1–11.
[8]. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol. 1966;22(6):764–79.
[9]. Bryne M, Koppang HS, Lilleng R, Kjærheim Å. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol. 1992;166(4):375–81.
[10]. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer. 2008;123(10):2229–38.
[11]. Dayan D, Hiss Y, Hirshberg A, Bubis J, Wolman M. Are the polarization colors of picrosirius red-stained collagen determined only by the diameter of the fibers? Histochemistry. 1989;93(1):27–9.
[12]. Hirshberg A, Sherman S, Buchner A, Dayan D. Collagen fibres in the wall of odontogenic keratocysts: a study with picrosirius red and polarizing microscopy. J Oral Pathol Med. 1999;28(9):410–2.
[13]. Junqueira L, Montes G, Sanchez E. The influence of tissue section thickness on the study of collagen by the Picrosirius-polarization method. Histochemistry. 1982;74(1):153–6.
[14]. Ceena DE, Bastian T, Ashok L, Annigeri RG. Comparative study of clinicofunctional staging of oral submucous fibrosis with qualitative analysis of collagen fibers under polarizing microscopy. Indian J Dent Res. 2009;20(3):271.
[15]. Ganganna K, Shetty P, Shroff SE. Collagen in histologic stages of oral submucous fibrosis: A polarizing microscopic study. J Oral Maxillofac Pathol JOMFP. 2012;16(2):162.
[16]. Johansson N, Airola K, Grénman R, Kariniemi A-L, Saarialho-Kere U, Kähäri V. Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. Am J Pathol. 1997;151(2):499.
[17]. Binnie W, Cawson R. A new ultrastructural finding in oral submucous fibrosis. Br J Dermatol. 1972;86(3):286–90.
[18]. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis–a collagen metabolic disorder. J Oral Pathol Med. 2005;34(6):321–8.
[19]. Canniff J, Harvey W. The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. Int J Oral Surg. 1980;10(Suppl 1):163–7.
[20]. Harvey W, Scutt A, Meghji S, Canniff J. Stimulation of human buccal mucosa fibroblasts in vitro by betel-nut alkaloids. Arch Oral Biol. 1986;31(1):45–9.
[21]. Allon I, Vered M, Buchner A, Dayan D. Stromal differences in salivary gland tumors of a common histopathogenesis but with different biological behavior: a study with picrosirius red and polarizing microscopy. Acta Histochem. 2006;108(4):259–64.
[22]. Monsky WL, Carreira CM, Tsuzuki Y, Gohongi T, Fukumura D, Jain RK. Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors. Clin Cancer Res. 2002;8(4):1008–13.
[23]. Koren R, Yaniv E, Kristt D, Shvero J, Veltman V, Grushko I, et al. Capsular collagen staining of follicular thyroid neoplasms by picrosirius red: role in differential diagnosis. Acta Histochem. 2001;103(2):151–7