Evaluation of fibrotic changes in OSMF: A retrospective study using special stains and polarizing microscopy

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ABSTRACT

Background: *Oral submucous fibrosis (OSMF), a potentially malignant oral disorder has the highest rate of malignant transformation of about 7-13%. The connective tissue changes that occur in this disease are characteristic and are stained with special stains.*

Objective: *The study was done to compare common and special stains under light microscopy and polarizing microscopy to evaluate the levels of fibrosis in oral submucous fibrosis and assess the type of collagen present in the stromal area.*

Materials and Methods: *Fifty tissue blocks were selected from the archives and were prepared and stained with H&E, Masson's trichrome, Van Gieson and Picrosirius red and studied under light microscope and polarizing microscope respectively.*

Results: *H and E stained slides were useful in diagnosing the lesion but was not able to highlight the level of fibrosis. Masson's trichrome and Van Gieson stained slides showed the depth of the lesion which extended even to the deeper muscle layer. The type of collagen present was definitively seen by the birefringence in polarizing microscopic study. Interobserver variation was less and all the values regarding the effectiveness of the special stains in detecting the level of fibrosis were statistically significant.*

Conclusion: *Special stains can be used routinely in laboratories to demonstrate connective tissue lesions especially in cases of OSMF. Depth of the lesion and the area of involvement help in treatment planning to be delivered. Large scale studies with more categories and inclusion criteria are required along with the special stains to assess the other alterations in OSMF.*

Key Words: OSMF, H & E, masson'strichrome, van gieson, picrosirius red, fibrosis, polarizing microscopy, collagen

Introduction

Fibrosis which is pathological is characterized by progressive and excessive accumulation of extracellular matrix collagen.It is an irreversible end stage of multitude diseases and can affect any organ in the body. Microscopically, fibrotic tissue is characterized by a loss of normal architecture, paucity of stromal cells, and replacement of blood vessels and other essential parenchymal structures by dense, homogeneous, and increasingly stable extracellular matrix. [1]The diseases where such pathological fibrosis occurs are oral submucous fibrosis, juvenile aggressive fibromatoses, and abdominal desmoids.[2]

Oral submucous fibrosis (OSMF) is defined as a chronic insidious disease affecting any part of the oral cavity and sometimes pharynx. Although it is 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 epithelial atrophy leading to stiffness of the oral mucosa causing trismus and inability to eat.[3,4] When compared to other diseases, OSMF is considered to be potentially harmful as it induces the overlying epithelium to undergo malignant transformation. Thus, OSMF is called as a precancerous condition. It has the highest malignant transformation rate when compared to other oral potential malignant disorders (OPMD'S) varying from 7-13%. [5,6]

Altered staining characteristics of the collagen have been helpful in demonstrating the typical histopathological features in OSMF. Although Haemotoxylinand Eosin(H and E) stain[7] is the most widely and commonly used histological stain in the diagnosis of oral submucous fibrosis, special stains such as Mallory[8], Masson's trichrome[9], Van gieson[10], Weigert'sresorcinfuchsin[11] have been used to demonstrate collagen in light microscopic studies in order to assess the level of fibrosis. The major disadvantage was the inability to demonstrate the type of collagen present in these lesions. This was demonstrated by Picrosirius red stain[12]and

the slides were studied using a polarizing microscope. [6,13]

The present study was undertaken to evaluate the levels of fibrosis in oral submucous fibrosis using HaemotoxylinandEosin and special stains such as Masson's Trichrome and Van Gieson's under light microscopy and Picrosirius red under polarizing microscopy to access the type of collagen present in the stromal area.

Materials and Methods

The study comprised of histopathologically proven cases of Oral submucous fibrosis, the details of which were retrieved from the records of Department of Oral and Maxillofacial Pathology, Thai Moogambigai Dental College and Hospital,Chennai from 2008 to 2014. Fifty tissue blocks were selected to study the distribution of various connective tissue fibres. The inclusion criteria for the study included detailed case records, the presence of epithelium in the biopsy slide, and presence of connective tissue fibers of sufficient depth to evaluate the levels of fibrosis

Paraffin embedded 4 µm thickness sections were prepared using semi-automatic microtome (Microm HM 340 e) for each case and subjected to routine hematoxylin and eosin stain[7] and special stains – Masson's Trichrome, [9]VanGieson's[10] and Picrosirius Red. [12,14] Standard staining protocol method was followed for all the 4 staining procedures. Fifty tissue blocks selected for the study were sectioned and stained with H& E, Masson's trichome, Van Gieson and were observed under the light microscope. Another set of slides were sectioned and stained with Picrosirius red and examined using the polarizing microscope. The slides were examined by 2 observers separately to avoid any bias and the interobserver variability was also assessed.

Results

Evaluation of the level of fibrosis was determined using Hand E stain by both the observers. 44% of fibrotic changes were seen in the superficial muscle fibers by observer 1 and 42% of fibrotic changes were noted by observer 2 {(Table 1) (Fig 1,2,3)}. Evaluation of the level of fibrosis was determined using Van giesons stain and was found that 46% of fibrotic changes were noted in the superficial muscle fibres by observer 1 and 50% of fibrotic changes were seen by observer 2 {(Table 2) (Fig 4,5,6)}.Evaluation of the levels of fibrosis was determined using Massonstrichrome stain by both the observers and found that 46% of fibrotic changes were noted in the superficial muscle fibres by observer 1 and 48% of fibrotic changes were seen by observer 2. In the deep muscle fibres, 48% of fibrotic changes were seen by observer 1 as compared to 46% seen by observer 2 {(Table 3) (Fig 7,8,9)}.

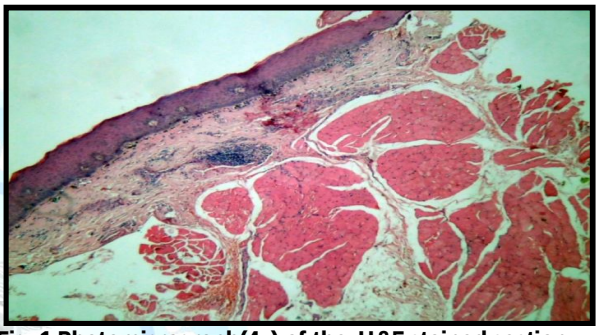

Fig. 1 Photomicrograph(4x) of the H&E stained section reveals Superficial Muscle Fiber Involvement with pink collagen fibres and nuclei appearing blue

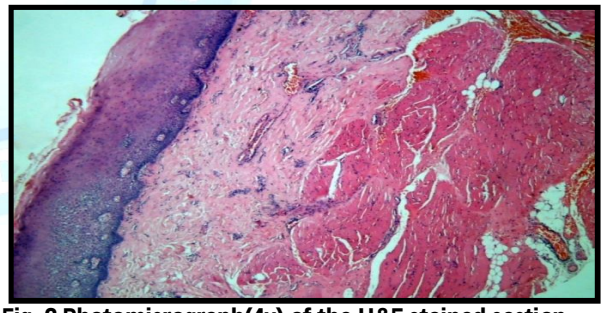

Fig. 2 Photomicrograph(4x) of the H&E stained section reveals Involvement of Lamina Propria with pink collagen fibres and nuclei appearing blue

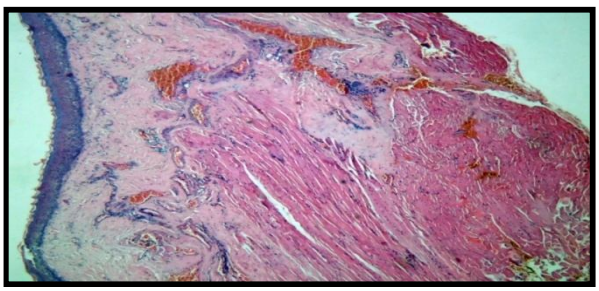

Fig. 3 Photomicrograph(4x) of the H&E stained section shows Deep Muscle Fiber Involvement with pink collagen fibres and nuclei appearing blue

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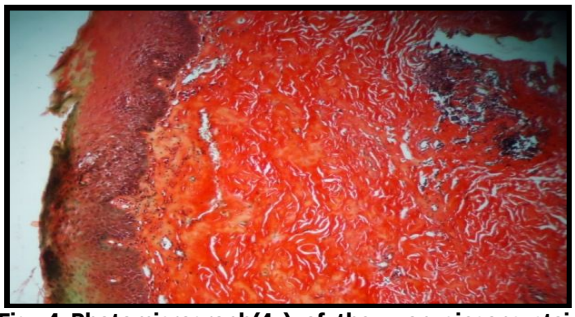

Fig. 4 Photomicrograph(4x) of the van giesons stained section shows lamina propria Involvement with orange red collagen fibres,cytoplasm appearing yellow in color & nuclei stained brown.

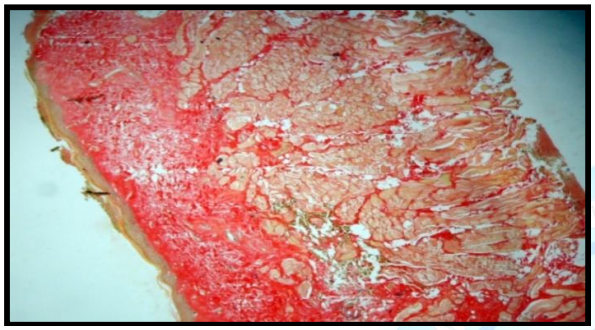

Fig. 5 Photomicrograph(4x) of the van giesons stained section shows Superficial Muscle fiber Involvement with deep red collagen fibres& nuclei stained brown

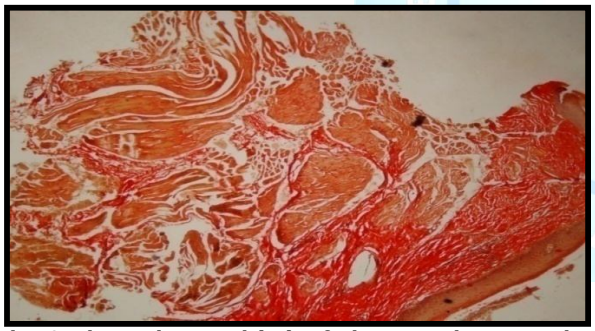

Fig. 6 Photomicrograph(4x) of the van giesons stained section shows Deep Muscle fiber Involvement with orange red collagen fibres& nuclei stained brown

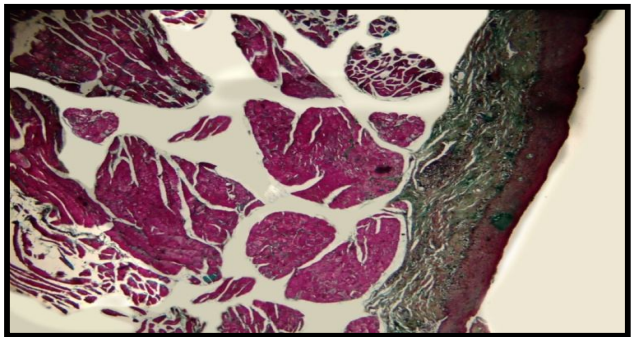

Fig. 7 Photomicrograph(4x) of the Massonstrichrome stained section reveals Lamina Propria Involvement with light green collagen fibres and nuclei stained black

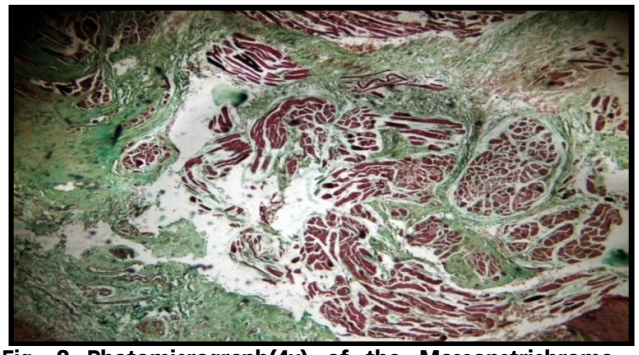

Fig. 8 Photomicrograph(4x) of the Massonstrichrome stained section reveals deep muscle fiber involvement with light green collagen fibres and nuclei stained black

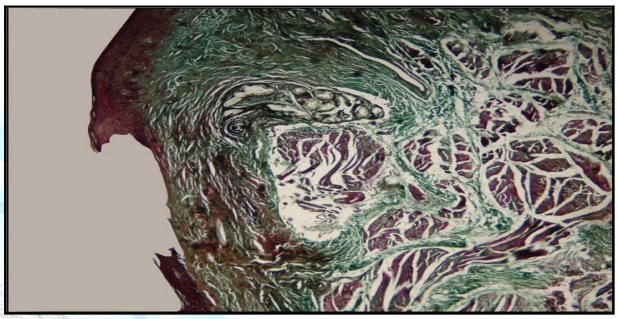

Fig. 9 Photomicrograph(4x) of the Massonstrichrome stained section shows lamina propria and Superficial muscle involvement with light green collagen fibres

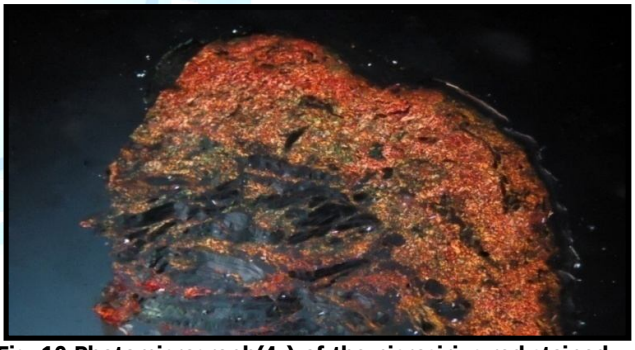

Fig. 10 Photomicrograph(4x) of the picrosirius red stained section under polarizing microscope shows Type I Collagen with orange red collagen fibres

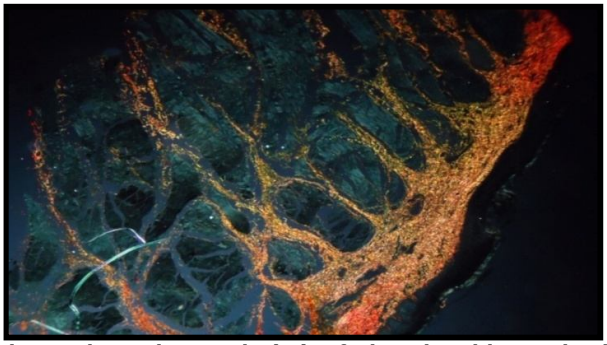

Fig.11 Photomicrograph (4x) of the picrosirius stained section under polarizing microscope shows Type III Collagen with dark green collagen

The interobserver variability of the effectiveness of Haematoxylin and Eosin stain was assessed and the variation in H and E stain effectiveness in superficial muscle fibers by observer 1&2 was found to be 17 & 22 and in deep muscle fibers it was 10 and 11 respectively. The p value was <0.001 and was statistically significant (Table 5). The interobserver variability of van gieson's stain effectiveness was assessed and the variation in the stain effectiveness as seen by observer 1 and 2 in superficial muscle fibres was 23 and 25 respectively and in deep muscle fibres was 24 and 22. The p value calculated was statistically significant (p<0.001) (Table 6)

The interobserver variability of Masson's trichrome stain effectiveness was assessed and the variation in the stain effectiveness by observer 1 and 2 in superficial muscle fibres was 23 & 24 and in deep muscle fibres was 24 and 23 respectively. The p value was <0.001 and found to be statistically significant (Table 7). The statistical analysis of chi-square test in comparing the interobserver variation of stain effectiveness shows subtle interobserver variation with p value < 0.001 which is statistically significant. Evaluation of the Type of Collagen was determined using Picrosirius red stain by both the observers show that 48% of samples show Type I collagen fibres in predominance and 52% of Type III collagen fibres as seen by observer 1. According to observer 2, Type I collagen fibres was predominantly observed in 44% sample and Type III collagen in 56% sample {(Table 4), (Fig 10,11)}.

Table 1: Evaluation of Level of Fibrosis Using H and E Stain, VanGiesons stain and Masson's trichrome stain and Inter observer variability

Method	Fibrosis level	N(%)	Interobserver variability
H and E Observer-1	Lamina propria only without involving deeper structures	17(34)	**Kappa value=0.721; p<0.001**
	Superficial muscle fibres	22(44)	
	Deep muscle fibres	11(22)	
H and E Observer-2	Lamina propria only without involving deeper structures	18(36)	
	Superficial muscle fibres	21(42)	
	Deep muscle fibres	11(22)	
VG Observer-1	Lamina propria only without involving deeper structures	3(6)	**Kappa value=0.856; p<0.001**
	Superficial muscle fibres	23(46)	
	Deep muscle fibres	24(48)	
VG Observer-2	Lamina propria only without involving deeper structures	3(6)	
	Superficial muscle fibres	25(50)	
	Deep muscle fibres	22(44)	
MT Observer-1	Lamina propria only without involving deeper structures	3(6)	**Kappa value=0.964; p<0.001**
	Superficial muscle fibres	23(46)	
	Deep muscle fibres	24(48)	
MT Observer-2	Lamina propria only without involving deeper structures	3(6)	
	Superficial muscle fibres	24(48)	
	Deep muscle fibres	23(46)	

Table 2: Evaluation of Type of Collagen Using Picrosirius Red Stain

Method	Fibrosis level	N	%
PSR Type Observer-1	Type –I	24	48.0
	Type –II	0	0
	Type –III	26	52.0
PSR Type Observer-2	Type –I	22	44.0
	Type –II	0	0
	Type –III	28	56.0

Table 3: Evaluation of stain effectiveness by observer 1

Staining Method	Level of Fibrosis:Obs-1							
	Lamina propria		Superficial muscle fibres		Deep muscle fibres		Total	
	N	Row %	N	Row %	N	Row %	N	Row %
H&E	17	34.0	22	44.0	11	22.0	50	100.0
VG	3	6.0	23	46.0	24	48.0	50	100.0
MT	3	6.0	23	46.0	24	48.0	50	100.0
PSR	1	2.0	23	46.0	26	52.0	50	100.0
Total	24	12.0	91	45.5	85	42.5	200	100.0

X^2=34.084; p<0.001

Table 4: Evaluation of stain effectiveness by observer 2

Staining Method	Level of Fibrosis:Obs-2							
	Lamina propria		Superficial muscle fibres		Deep muscle fibres		Total	
	N	Row %	N	Row %	N	Row %	N	Row %
H&E	18	36.0	21	42.0	11	22.0	50	100.0
VG	3	6.0	25	50.0	22	44.0	50	100.0
MT	3	6.0	24	48.0	23	46.0	50	100.0
PSR	1	2.0	21	42.0	28	56.0	50	100.0
Total	25	12.5	91	45.5	84	42.0	200	100.0

X^2=37.774; p<0.001

Discussion

OSMF is a very chronic, potentially malignant condition of the oral cavity which predominantly occurs in India and South East Asia. [4] The present study was designed to determine the level of fibrotic changes using van gieson's stain and Masson's trichrome stain and to determine the type of collagen involved in OSMF using Picrosirius red stain. In this study, the fibrosis is more prominently seen in superficial muscle layer using Haematoxylin and Eosin(44%) Van gieson's(46%) and Masson's trichrome(46%). Observation of observer I is in par with observer II which is Haematoxylin and Eosin (42%), Van gieson's(50%), Masson's trichrome(48%), respectively(Table 1,2,3,5,6,8). H and E staining which is the most commonly used stain in pathology, highlighted the fibrotic changes at the deeper muscle fiber level in very few samples when compared to special stains such as Van Giesonsand Masson's trichrome which revealed

fibrotic changes even in the lamina propria level (Table 1,2,3). Therefore special stains are found to be more efficient than H and Ein assessing fibrosis ($p<0.01$) which is in correlation with the study by Rooban et al., 2005 (15).The fibrosis was present in the superficial muscle fibers(40%) and also in the deeper muscle region(10%). The study of the level of fibrosis using H and E and special stains using Light Microscope was similar to the studies by Gupta et al, [5] and Joseph et al. [10] Polarizing microscopy was used to study the type of collagen using picrosirius red stain showed that Type I & Type III collagen types are seen more in majority of cases by both the observerswith a significant p value (< 0.01). The finding of the present study was in line with the study by Kamath et al, [16,17]who correlated the type of collagen with the functional and histological grading.

In our study, an attempt has been made to qualitatively assess the staining patterns of three stains (H and E, Vangiesonsand Massonstrichrome) and the predominant type of collagen by picrosirius red stain. Special stains were found to be useful in identifying the levels of fibrosis and should be routinely used in labs along with H and E. The type of collagen present which was reported by the study using picrosirius red stain and polarizing microscope suggests the sites where the fibrosis is initiated and the composition of collagen that is obtained. It is important to know the location of fibrosis as this can serve as a guideline for initiating treatment. The risk factors associated with the development of cancer in OSMF needs to be understood. [18]In some cases the submucosal layer can show fibrosis prior to lamina propria involvement in the early stage of OSMF. [2] In such cases the clinical manifestations of xerostomia[19] leading to candidal colonization, [20,21] decay, early muscular involvement leading to reduction in mouth opening, alterations in the epithelium can occur leading to lethal consequences. Thus, early identification of the level of fibrosis is important in initiating treatment as OSMF leads to irreversible changes in the oral mucosa. When considering the type of collagen the chemical composition varies between the different types thus requiring different methods to stop the fibrotic process. This influences the therapeutic options that need to be used for the cases.

In this study, the fibrosis was predominantly seen in the superficial muscle layer using Haematoxylin & Eosin. Special stains like Van gieson's and Masson's Trichrome revealed deeper areas of fibrosis. Polarizing microscopy using picrosirius red stain showed that Type I and Type III collagen types were predominant. No significant variation was observed in the assessment by both the observers. The fact that special stains are superior in assessing the fibrosis in Oral Submucous Fibrosis was established by the study. Future studies are required along these lines including parameters such as Clinical & Histopathological correlation, orientation of fibers, degeneration of muscle fibers, epithelial changes, vasculometric analysis with more number of observers and larger sample size. This study highlights the fact that routine use of special stains in laboratory can be useful in the planning of early intervention in early stage OSMF and reduce the morbidity and mortality for the patient.

References

1. Varga J, Brenner D, Phan SH. Fibrosis research methods and protocols. Available at. www.springer.com/productFlyer_978-1-58829 -479-1.pdf?SGWID=0-0-1297...0
2. Joseph AP, Rajendran R. Submucosa precedes lamina propria in initiating fibrosis in oral submucous fibrosis-evidence based on collagen histochemistry. J Oral Maxillofac Pathol 2010;1:3-11.
3. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764–79.
4. Reddy V, Wanjari PV. Oral submucous fibrosis: Correlation of clinical grading to various habit factors. Int J Dent Clin 2011;3:21–4.
5. Gupta MK, Shubangi Mhaske, Raju Raghavendra. Oral submucous fibrosis-current concepts in Etiopathogenesis. Peoples journal of scientific research2008;1:39-44.

6. Rajpal K, Grover Neeraj, Sengupta S, Sangeeth singh, Nishant singh, Paramjit singh, et al. Qualitative comparison of various stains to assess the tinctorial properties of collagen in Oral submucous fibrosis. Journal of orofacial and health sciences 2013;4(2):76-8.
7. Stevens A, Bancroft John D, Gamble M. The haematoxylins. Theory and practice of histological techniques. 3rd ed. London: Churchill Livingstone; 1990.p.107-17.
8. Mallory FB. The anilin blue collagen stain. Biotechnic & Histochemistry 1936;11(3):101-2.
9. Masson P, Claude L. Some histological methods. Trichrome stainings and their preliminary technique. J Tech Methods 1929;12:75-90.
10. Van Gieson. Laboratory notes of technical methods for the nervous system. Publisher not identified.1889.
11. Clark G, Pennington RC. Weigert's Resorcin Fuchsin. Journal of Histotechnology 1982;5(2):69-70.
12. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. The Histochemical journal 2011;(4):447-55.
13. Ceena DE, Bastian TS, Ashok L, AnnigeriRG.Comparative study of clinic functional staging of oral submucous fibrosis with qualitative analysis of collagen fibers under polarizing microscopy. Indian journal of dental research2009;20(3):271-6.
14. Puchtler H, Waldrop FS, Valentine LS. Polarization microscopic studies of connective tissue stained with picro-siriusred. FBA. BeitragezurPathologie 1973;150(2):174-87.
15. Rooban T, Saraswathi TR, Fathima al zainab, Uma Devi, Joshua Elizabeth, Ranganathan K. A light microscopic study of fibrosis involving muscle in oral submucous fibrosis. IJDR 2005;16(4):131-4.
16. Kamath VV. The nature of collagen in oral submucous fibrosis: A systematic review of the literature. Saudi J Oral Sci 2014;1:57-64.
17. Kamath VV, Satelur K, Komali Y, Krishnamurthy SS. Image analysis of collagen types and thickness in oral submucous fibrosis stained with picrosirius red under polarizing microscope. Journal of orofacial sciences 2013;5(2):123-7.
18. Pillai R, Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis: Relationship to risk factors associated with oral cancer. Cancer 1992;69(8):2011-20.
19. Nyachhyon R, Boaz K, Sumanth KN. Minor salivary gland changes in oral submucous fibrosis (OSMF): Retrospective pilot study. Journal of Nepal Dental Association 2011;12(1):26-8.
20. Sharma P, SaxenaS. Candida albicans and its correlation with oral epithelial neoplasia. International Journal of Oral-Medical Sciences2011;10(3):140-8.
21. Kamat MS, VanakiSS, Puranik RS, Puranik SR, Kaur R. Oral Candida carriage, quantification, and species characterization in oral submucous fibrosis patients and healthy individuals. Journal of investigative and clinical dentistry2011;2(4):275-9.

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