Original Article

Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma

Bhari Sharanesha Manjunatha, Aditi Agrawal[1], Vandana Shah[2]

Department of Dental Anatomy and Oral Biology, Faculty of Dentistry, Taif University, Taif, Kingdom of Saudi Arabia, [1]Department of Oral Pathology, Ahmedabad Dental College and Hospital, Near Science City, Ahmedabad, [2]Department of Oral Pathology, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India

For correspondence:
Dr. Bhari Sharanesha Manjunatha, Faculty of Dentistry, Taif University, Taif, Kingdom of Saudi Arabia.
E-mail: drmanju26@hotmail.com

ABSTRACT

Background: Collagen is a part of structural connective tissue in every organ of the body. Study of collagen brings into focus the current knowledge of biosynthesis and degradation as a complex mechanism. One of the major aspects of tumor cell invasion and metastasis is the interaction between cancer cells and extracellular matrix component. Property of picrosirius red to enhance the birefringence is the result of basic protein nature of collagen molecules. There exists a direct relationship between the presence of type IV collagen and degree of differentiation in squamous cell carcinoma (SCC) cells as they lose their capability to form the basement membrane.

Materials and Methods: Thirty cases of oral SCC (OSCC) were evaluated for standard staining protocol of picrosirius red to analyze collagen fibers in different grades of OSCC under polarizing microscopy.

Results: In this study, moderately to poorly differentiated OSCC cases showed a gradual change in polarizing colors from yellowish orange to greenish yellow particularly in the vicinity of invading tumor islands. Thick collagen fibers forming bundles in parallel disposition were found around the neoplastic areas in discontinuous fashion. Type I collagen fibers of different lengths were strongly birefringent and swirl pattern, parallel orientation was evident.

Conclusion: An observable stromal change with the progression of neoplasm was evinced with picrosirius red stain in different thickness of collagens with a significant change in the arrangement from the early stage to the advanced stage according to tumorogenesis.

KEY WORDS: Collagen, extracellular matrix, oral squamous cell carcinoma, picrosirius red stain, polarizing microscopy, tumorogenesis

INTRODUCTION

Basement membranes are complex structures composed of a mixture of collagen, glycoproteins, proteoglycans.[1] Four major molecules are present in most basement membranes: Type IV collagen, laminin, perlecan, and entactin.[2] Neoplastic invasion and metastases are characterized by the ability of tumor cells to cross tissue compartment boundaries. The subepithelial basement membrane plays an important role in the complex interactions of this process, as it is the first obstacle to be traversed by the neoplastic cells. The extracellular microenvironment of tumors is determined by the matrix synthesized by normal and tumor cells, as well as the host stromal components secreted by surrounding host fibroblasts. Even in a single tumor there may be variations in the stroma, from one area to another, and composition of the stroma may evolve over time.[3]

Carcinomas are characterized by invasion of malignant cells into the underlying connective tissue and migration of malignant cells to form metastases at distant sites. These processes require alterations in cell–cell and cell–extracellular matrix (ECM) interactions.[4] Loss of basement membrane has been associated with many types of carcinomas, with tumor cells in lymph nodes and organ metastases.[5]

Role of fibrous components (collagen, elastin and reticulin fibers) and ground substance (glycoproteins, mucins and fibrin) in different stages of neoplasia remains to be defined. The reactive changes in the tumor stroma may alter the biological aggressiveness of oral cancer.[6]

Picrosirius red stain is considered a highly specific and selective stain for collagen fibers due to its ability in differentiating between different types of collagen fibers in various pathological conditions.[7]

Access this article online
Website: www.cancerjournal.net
DOI: 10.4103/0973-1482.154061
Quick Response Code:

This study was undertaken to evaluate the collagen fibers using picrosirius red stain and polarizing microscopy and to compare the nature and orientation of collagen fibers in various grades of the thickness and their molecular packing. Hence, this property of collagen can be used for the purpose of its quantitative and qualitative analysis.

MATERIALS AND METHODS

Thirty blocks of oral squamous cell carcinoma (OSCC) were retrieved from the archives of Department of Oral and Maxillofacial Pathology, during 2011–2014 and evaluated. Formalin fixed paraffin embedded tissue blocks were obtained as source of material for the study. Tissue sections of about 5 μ were stained first with hematoxylin and eosin for histopathological confirmation of the section and after with picrosirius red stain. A standard protocol staining of picrosirius red was used, and sections were observed under polarizing microscopy for orientation, arrangement, nature of collagen fibers in different grades of OSCC. The sections were further graded according to the World Health Organization grading system, revised in 2005[8] based on Broders' classification[9] which looks for microscopic differences between normal epithelium and tumoral tissue because of a lack of cellular differentiation. This classification has applications in routine anatomic pathology for analysis of biopsy and surgical specimens. Currently, it is also based on the degree of cell differentiation, grouping SCC into three categories: Well-moderately and poorly differentiated tumors.[8]

Collagen in various grades of OSCC were analyzed quantitatively and speculated that that in the invasive front of cancer tissue behaving inconsistence in spacing, increased thickness and disruption of normal collagen as stromal reaction creates an area suitable for invasive cancer growth and also provides a host defense mechanism that prevents malignant access into the blood stream. A recent study by Li *et al*. in 2013 mentions that collagen fiber plays an important role in ECM destruction and remodeling. Collagen fiber content in OSCC tissue significantly correlated with tumor differentiation, recurrence and nodal status reflecting the key role of it in the carcinogenic process.[10] When observed polarizing colors of collagen fibers changed gradually from reddish orange to greenish yellow from well to poorly differentiated SCC, thus indicating that as the tumor progresses, there is a change from mature collagen to an immature collagen form.

RESULTS

It was also observed that interstitial collagen displayed a different birefringence of colors with different intensities.

Table 1 and Figure 1a-c exhibits collagen fibers around the tumor islands with reddish orange and yellowish orange birefringence in well to moderate differentiated OSCC, poorly differentiated OSCC as yellowish green birefringency.

Table 2 and Figure 2a-c exhibit collagen fibers as swirls in well-differentiated carcinoma and moderately differentiated SCC, poorly differentiated SCC in bundles along with cross hatchet arrangement.

Table 3 and Figure 3 exhibit collagen fibers in parallel orientation in all grades of OSCC. Table 4 and Figure 4a and b exhibit collagen fibers as strong and weak birefriency in well to poorly differentiated SCC. Figures 5-8 shows bar charts.

Yellow green birefringency was evident in 1c in poorly differentiated form. In statistical analysis of nature of collagen fibers under polarizing microscopy showed significant birefringency with Chi-square value 14.319, $P < 0.005$.

DISCUSSION

In the study the collagen fibers are reticular/fibrillar and more disorganized in moderately to poorly differentiated

Table 1: Polarizing colors observed in three grades of OSCC

Color	Grading of OSCC (%)			Total (%)
	Well	Moderate	Poor	
Greenish yellow	2 (40)	2 (40)	1 (20)	5 (100)
Yellowish orange	1 (14.28)	4 (57.14)	2 (28.57)	7 (100)
Reddish orange	8 (44.44)	8 (44.44)	2 (11.11)	18 (100)
	11 (36.66)	14 (46.66)	5 (16.66)	30 (100)

OSCC=Oral squamous cell carcinoma

Table 2: Histopathological correlation of collagen fibers arrangement in OSCC under polarizing microscopy

Arrangement	Grading of OSCC (%)			Total (%)
	Well differentiated	Moderate differentiated	Poorly differentiated	
Bundles	2 (33.33)	3 (50)	1 (16.67)	6 (100)
Cross hatchet	2 (33.33)	3 (50)	1 (16.67)	6 (100)
Swirls	7 (38.89)	8 (44.45)	3 (16.66)	18 (100)
	11 (36.67)	14 (46.66)	5 (16.67)	30 (100)

OSCC=Oral squamous cell carcinoma

Table 3: Orientation of collagen fibers observed in OSCC under polarizing microscopy

Orientation	Grading of OSCC (%)			Total (%)
	Well differentiated	Moderate differentiated	Poorly differentiated	
Parallel	9 (39.13)	10 (43.48)	4 (17.39)	23 (100)
Not parallel	2 (28.58)	4 (57.14)	1 (14.28)	7 (100)

OSCC=Oral squamous cell carcinoma

Table 4: Nature of collagen fibers under polarizing microscopy in OSCC

Nature of birefringency	Grading of OSCC (%)			Total (%)
	Well differentiated	Moderate differentiated	Poorly differentiated	
Strong	10 (45.45)	11 (50)	1 (4.55)	22 (100)
Moderate	1 (50)	1 (50)	0 (0)	2 (100)
Weak	0 (0)	2 (33.33)	4 (66.67)	6 (100)
	11 (36.67)	14 (46.67)	5 (15.66)	30 (100)

OSCC=Oral squamous cell carcinoma

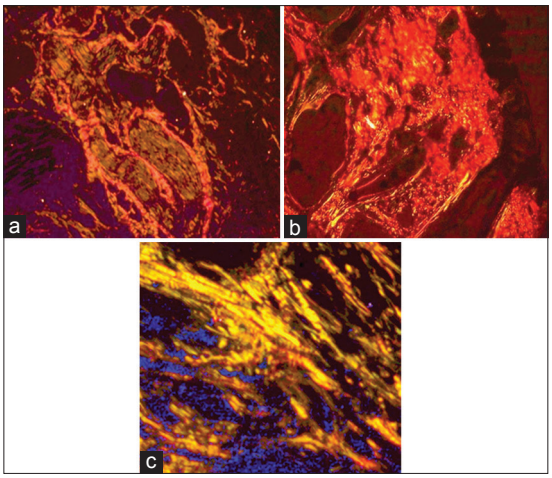

Figure 1: (a) Photomicrograph showing collagen fibers in oral squamous cell carcinoma with reddish orange to yellowish color under polarizing microscopy in ×10. (b) Photomicrograph of collagen fibers in oral squamous cell carcinoma of yellowish red to orangish red color under polarizing microscopy in ×10. (c) Photomicrograph showing collagen fibers in oral squamous cell carcinoma with yellow-green birefringence under polarizing microscopy

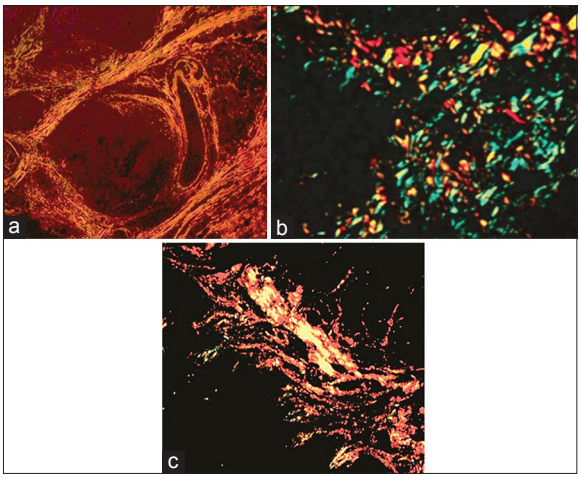

Figure 2: (a) Photomicrograph of collagen fibers in oral squamous cell carcinoma with cross hatchet arrangement under polarizing microscopy. (b) Photomicrograph of collagen fibers in oral squamous cell carcinoma with bundle arrangement under polarizing microscopy. (c) Photomicrograph of collagen fibers in oral squamous cell carcinoma with swirls arrangement under polarizing microscopy

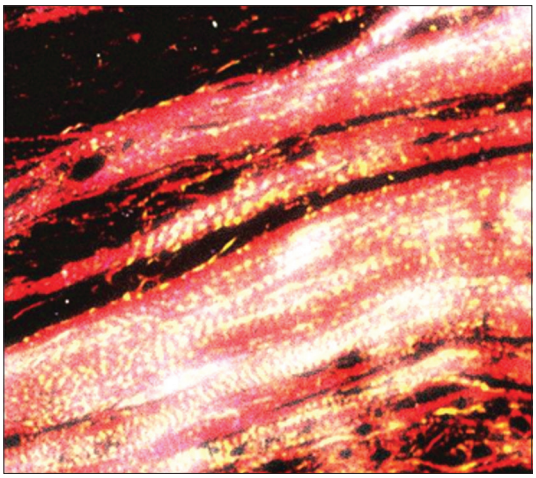

Figure 3: Photomicrograph showing collagen fibers in oral squamous cell carcinoma with parallel orientation under polarizing microscopy

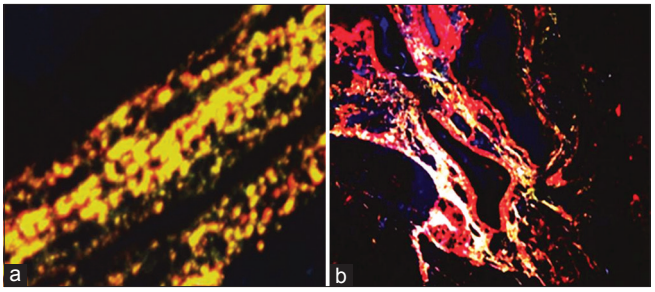

Figure 4: (a) Photomicrograph of collagen fibers in oral squamous cell carcinoma with weak birefringence under polarizing microscopy. (b) Photomicrograph showing collagen fibers in oral squamous cell carcinoma with red strong birefriency under polarizing microscopy

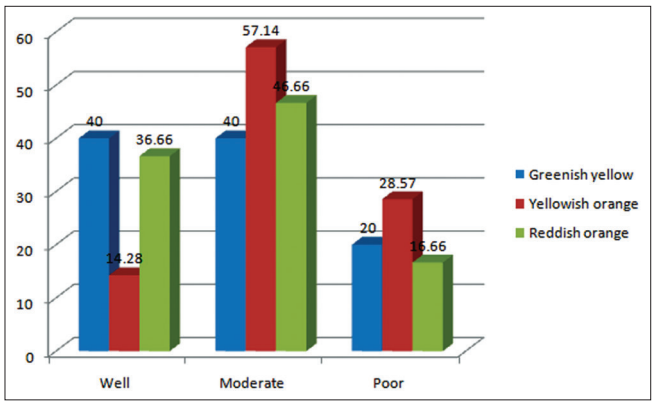

Figure 5: Bar chart showing polarizing colors of collagen fibers in different grades of oral squamous cell carcinoma

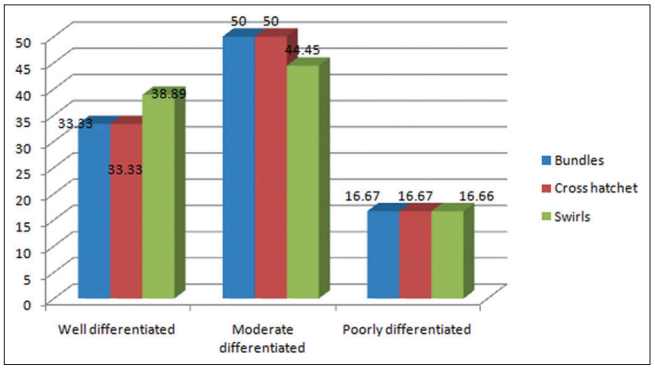

Figure 6: Bar chart showing histopathological correlation of collagen fibers arrangement in oral squamous cell carcinoma

SCC indicating contribution of stroma constituents in the progression of the neoplasm as stromal destruction may enhance the movement of the tumor cells toward the blood vessels or the lymphatic vessels. These observations in the study indicated stromal changes at the invading front of tumor islands helps in predicting tumor behavior and birefringence

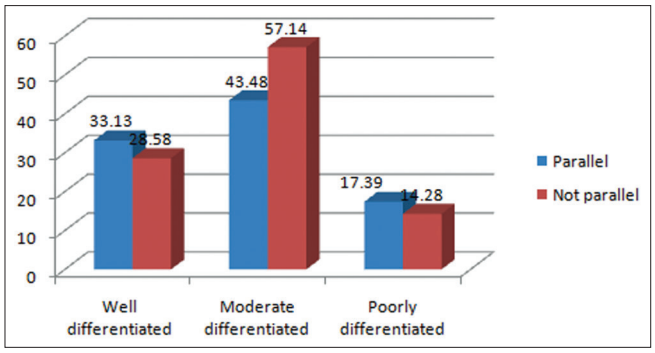

Figure 7: Bar chart showing orientation of collagen fibers in oral squamous cell carcinoma

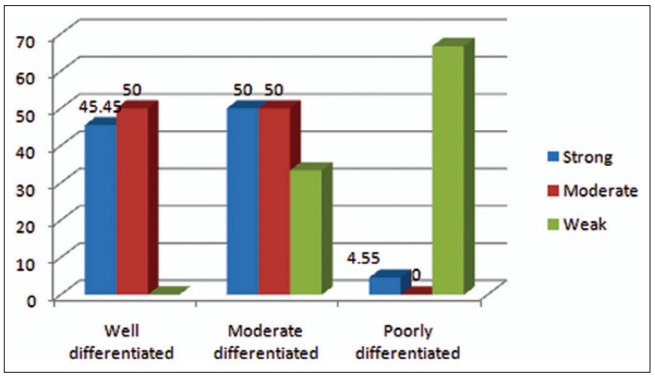

Figure 8: Bar chart showing nature of collagen fibers in oral squamous cell carcinoma

fibers in picrosirius red stained section enabled to differentiate between compactness of collagen by change in polarizing colors. In arrangement of collagen fibers, advanced stages of OSCC showed mature collagen to immature form.

In addition, Junqueira *et al.*,[11] stated under pathological condition birefringence showed a different pattern in comparison with collagen in normal tissue and proved that type I collagen were thick, strongly birefringent red fibers while type III collagen appeared as thin weakly birefringent green fiber and thus supporting our study.

The polarizing colors of collagen fibers could be due to various growth factors and cytokines that cause proliferation of fibroblasts and ECM resulting in the formation of thick mature collagen. As collagen matures, the change in proteoglycans content of fiber causes dehydration of the fibers thereby, increasing the diameter of collagen fibers. Thus, to tight packing of collagen, there was the difference in polarizing colors.[12]

Oral squamous cell carcinoma is showing local invasion of the underlying connective tissue in forms of islets and cords of epithelial cells. Interaction between tumor cells and ECM components was essential for tumor growth and onset of cell spreading and subsequent metastatic activity. The stromal tissue in breast cancer has also shown that increase in the collagen content of the ECM increasing mechanical stiffness and transport resistance of the tumor.[13-16]

Thus, authors reported remnants of basement membrane material may represent areas of partial regression of the neoplasm establishing these noncohesive irregular small cords, and single cells were more likely to attain access to the vasculature and develop metastasis. Thus, invasive front should always be the field of study of alterations in the basement membrane in OSCC.[17]

In study done by, Allon *et al.*[18] found stromal differences in salivary gland tumors, 50% of collagen fibres in polymorphous low-grade adenocarcinoma and adenoid cystic carcinomas were greenish yellow, whereas in pleomorphic adenoma, only 13% of them were greenish yellow.

CONCLUSION

Our results are consistent with the study done by Venigella and Charu[19] with gradual change in polarizing colors along with advancing grade of the tumor from yellowish orange to greenish yellow showing tumor progression.

REFERENCES

1. Elwood JM, Pearson JC, Skippen DH, Jackson SM. Alcohol, smoking, social and occupational factors in the aetiology of cancer of the oral cavity, pharynx and larynx. Int J Cancer 1984;34:603-12.
2. d'Ardenne AJ. Use of basement membrane markers in tumour diagnosis. J Clin Pathol 1989;42:449-57.
3. Nagy JA, Brown LF, Senger DR, Lanir N, Van de Water L, Dvorak AM, *et al.* Pathogenesis of tumor stroma generation: A critical role for leaky blood vessels and fibrin deposition. Biochim Biophys Acta 1989;948:305-26.
4. Lyons AJ, Jones J. Cell adhesion molecules, the extracellular matrix and oral squamous carcinoma. Int J Oral Maxillofac Surg 2007;36:671-9.
5. Matsumoto K, Horikoshi M, Rikimaru K, Enomoto S. A study of an *in vitro* model for invasion of oral squamous cell carcinoma. J Oral Pathol Med 1989;18:498-501.
6. George J, Narang RS, Rao NN. Stromal response in different histological grades of oral squamous cell carcinoma: A histochemical study. Indian J Dent Res 2012;23:842.
7. Rich L, Whittaker P. Collagen and picrosirius red staining. A polarised light assessment of fibrillar hue and spatial distribution. Braz J Morphol Sci 2005;22:997-1004.
8. Barnes L, Eveson JW, Riechert P, Sidransky D, editors. World Health Organisation Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005. p. 140-70.
9. Broders AC. The microscopic grading of cancer. Surg Clin North Am 1941;21:947-62.
10. Li HX, Zheng JH, Fan HX, Li HP, Gao ZX, Chen D. Expression of avß6 integrin and collagen fibre in oral squamous cell carcinoma: Association with clinical outcomes and prognostic implications. J Oral Pathol Med 2013;42:547-56.
11. Junqueira LC, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Arch Histol Jpn 1978;41:267-74.
12. Singh HP, Shetty DC, Wadhwan V, Aggarwal P. A quantitative and qualitative comparative analysis of collagen fibers to determine the role of connective tissue stroma on biological behavior of odontogenic cysts: A histochemical study. Natl J Maxillofac Surg 2012;3:15-20.

13. Ghosh S, Munshi HG, Sen R, Linz-McGillem LA, Goldman RD, Lorch J, *et al.* Loss of adhesion-regulated proteinase production is correlated with invasive activity in oral squamous cell carcinoma. Cancer 2002;95:2524-33.
14. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353-64.
15. Perez-Ordoñez B, Beauchemin M, Jordan RC. Molecular biology of squamous cell carcinoma of the head and neck. J Clin Pathol 2006;59:445-53.
16. Monsky WL, Mouta Carreira C, 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;8:1008-13.
17. Van Cauwenberge D, Pierard GE, Foidart JM, Lapiere CM. Immunohistochemical localization of laminin, type IV and type V collagen in basal cell carcinoma. Br J Dermatol 1983;108:163-70.
18. 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;108:259-64.
19. Venigella A, Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the picrosirius red stain: Histochemical study. J Clin Diagn Res 2010;4:3444-9.

Cite this article as: Manjunatha BS, Agrawal A, Shah V. Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma. J Can Res Ther 2015;11:272-6.

Source of Support: Nil, **Conflict of Interest:** None declared.