Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 9, 4557-4560

Qualitative Analysis Of Stromal Collagen In Different Grades Of Oral Squamous Cell Carcinoma Using Special Stains

Dr. Roli Singh[1]*, Dr. Vijay Wadhwan[2], Dr. Vandana Reddy[3]

*[1]*Assistant Professor, Department of Oral and Maxillofacial Pathology & Oral Microbiology, Subharti Dental College & Hospital, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh. Email: rolisingh786@gmail.com*

[2]*Professor & Head , Department of Oral and Maxillofacial Pathology & Oral Microbiology, Subharti Dental College & Hospital, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh.*

[3]*Professor, Department of Oral and Maxillofacial Pathology & Oral Microbiology, Subharti Dental College & Hospital, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh.*

*Corresponding Author: Dr. Roli Singh

Abstract

Introduction: Oral cancer constitutes as a major health issue in developing countries, representing the leading cause of death. The characteristics of the stroma in relation to the invading malignant epithelial cells and the interdependence between the stroma and the tumor cells are always a matter of discussion and interest.
Aim: To evaluate and compare the changes of stromal collagen fibres in different grades of Oral Squamous Cell Carcinoma (OSCC) sections stained with Masson's Trichrome, Van Gieson & Picrosirius Red stain and compare with normal oral mucosa.
Materials & Methods: A total of 40 histologically proven cases comprising 10 well differentiated OSCC, 10 moderately differentiated OSCC, 10 poorly differentiated OSCC and 10 cases normal oral mucosa were examined for the changes in collagen fibres by using special stains (Van Gieson, Masson Trichrome, and Picrosirius Red). Interpretation of staining intensity was carried out and statistically analysed.
Results: It was observed that there was gradual change in staining intensity of stromal collagen fibers from well to poorly differentiated OSCC sections stained with special stains.
Conclusion: The observation of study revealed that altered staining intensity of collagen fibres may indicate their role in tumour invasion which interprets that tumour cells are capable of manipulating the extracellular matrix resulting in destruction of surrounding stroma.

Keywords: OSCC, Special stains, Collagen fibres, Stroma.

Introduction:

OSCC is a major public health issue in India, accounting for 30% of all cancers and showing a troubling increase in incidence among both sexes, with 1,30,000 people dying from the disease each year, equating to approximately 14 deaths per hour[1]. Traditional diagnostic methods such as clinical assessment, histopathological examination and imaging techniques are limited in their capacity to provide information on prognosis and treatment of choice for head and neck cancer [2, 3]. Approximately 90% of deaths due to oral cancer are caused by metastasis. Despite advances in cancer diagnosis and treatment, the survival rate for oral cancer (around 50%) has remained unchanged over the last few decades [4].

Squamous cell carcinomas are malignant epithelial neoplasms showing atypically arranged epithelial cells with varying degrees of differentiation [5]. These oncogenetically mutated cells invade through the basement membrane causing changes in tumor microenvironment (TME), which can profoundly be seen as reactive changes in the stroma which may alter biological aggressiveness of oral cancer [6]. ECM is composed of network of ground substance, collagen and elastin embedded in a viscoelastic ground substance composed of proteoglycans and glycoproteins which are the versatile and dynamic aspects of cell biology [7]. Collagen is abundant protein present in ECM which helps in maintaining the structural integrity and tissue function. Collagen is thought to be the primary barrier that must be breached during invasion in order for the tumour mass to infiltrate [8].

Special stains being less expensive, easily available and less time consuming than immunohistochemistry

can be routinely used to understand the stromal changes in oral cancers. They make the analysis easy as well as less erroneous by highlighting the area of interest [9]. However, there is paucity of studies on the role of collagen fibres in different grades of oral cancer and still remains to be defined in the Indian subcontinent. Incorporation of methods to detect and evaluate the connective tissue stroma into prognostic system may help to see the biological diversity of oral cancer and thus to predict outcomes [8]. Thus, the purpose of the present study was to analyse the morphological changes in collagen fibres in different grades of OSCC and to know the influence of these changes in predicting the biological behaviour.

Further the study also assessed:-

1. The birefringence of collagen fibers in different grades of OSCC and normal oral mucosa using Picrosirius Red stain with polarizing microscope.
2. The staining intensity of collagen in different grades of OSCC and normal oral mucosa using Van Gieson stain with light microscope.
3. The staining intensity of collagen in different grades of OSCC and normal oral mucosa using Masson Trichrome stain with light microscope.

Material and Methods:

This crossectional study was conducted on the archival retrieved formalin fixed, paraffin-embedded tissue blocks which were obtained from the Department of Oral and Maxillofacial Pathology, Subharti Dental College, Meerut. The study groups included histological sections of previously diagnosed cases of OSCC based on Broder's histological grading [10] and control tissue specimens of normal oral mucosa. Cases with original diagnostic slides which have large enough tumor tissue were included in the study. These cases comprised of 10 cases of well differentiated OSCC (Group I), 10 cases of moderately differentiated OSCC (Group II), 10 cases of poorly differentiated OSCC (Group III) and 10 cases of normal oral mucosa (Group IV).

3 sections each of 4 µ thickness, from paraffin embedded blocks from each sample were prepared. 2 sections were stained with Van Gieson and Masson Trichrome and observed under the light microscope for morphological changes in collagen based on staining intensity around tumor islands in OSCC. All the cases of normal oral mucosa were also treated with these staining. At a magnification of X100, the images in the five chosen fields were evaluated. Strongly positive= 2, mildly positive= 1, weakly positive/negative= 0 were the staining intensities around the tumour islands [11]. PSR was used to stain the third section, which was examined using a polarising light microscope. The color of collagen fibre birefringence was analysed in five randomly selected areas in each case at X100 magnification around the tumor islands in squamous cell carcinoma and in lamina propria in normal oral mucosa. Predominant birefringence of collagen fibres was noted as reddish orange (RO), yellowish orange (YO) and greenish yellow (GY) [3].

Results were statistically analysed using SPSS statistics software. Correlation of the staining intensity and birefringence of collagen fibres in all three grades of OSCC was studied by using Fischer exact test of association and Z test respectively.

Results:

In the current study, Collagen fibre staining intensity in well-differentiated OSCC cases and normal oral mucosa revealed strongly positive staining due to abundant thick collagen fibres around tumour islands. Moderately differentiated OSCC cases showed mild positive staining intensity. Poorly differentiated OSCC cases showed thin weakly stained few dispersed collagen fibres. On application of Fischer exact test significant 'p' value was noted in staining intensity of collagen fibres with van Gieson and Masson trichrome (p value= 0.0063 and p value= 0.0041) in all the different grades of OSCCs and normal oral mucosa.

When section were stained with Picrosirius Red stain and observed under Polarizing microscope, collagen fibres around tumour islands in the majority of fields of normal oral mucosa and well differentiated OSCC showed polarising colours of Reddish Orange. In most of the cases of moderately differentiated OSCC the fibres predominantly exhibited a yellowish orange birefringence and in majority of poorly differentiated OSCC cases, the fibres predominantly exhibited a greenish yellow birefringence. Comparison between the nature of collagen in different grades of OSCC showed that the difference was statistically significant (p value= 0.0056) between all the grades of OSCC and normal oral mucosa.

Discussion:

Squamous cell carcinoma (SCC) is the most frequent malignancy of the oral cavity which is known to have high mortality rate [7]. Apart from habits such as tobacco, alcohol and betel quid chewing, other

etiological factors can be poor oral hygiene, ill-fitting prosthesis and chronic irritation. In addition, infection by human papillomavirus is an important causative factor in oropharyngeal carcinoma. The median age at the time of diagnosis of OSCC is sixth decade, and males are predominantly affected [11].

SCC consists of two discrete interdependent components- the tumor epithelial cells themselves and the stroma in which they are dispersed [12]. It has been reported that tissue produced by the altered cells differs from that produced by the normal cells as the invading tumor cells induces abundant collagenous or desmoplastic stroma [13]. The stromal collagen fibers can benefit the tumor by reducing access to the host lymphocytes or these fibers can benefit the host by walling off the invading tumor [14]. Earlier studies [15, 13] suggest that thickness of collagen fibers decrease with progressing OSCC. This can be explained as that in a disease condition such as cancer, there is persistent secretion of collagen- destroying enzymes (collagenases, proteinases) by cancer cells causing the destruction of the surrounding collagen. As a result, the surrounding collagen fibers are dissolved or immature, facilitating tumor growth, and metastasis.

The results of our study were consistent with the findings of Patankar SR et al [11], Khan S et al [15] who showed similar staining intensity of collagen fibers in different grades of OSCC. George et al [5] in their study evaluated the response of the stroma in varying grades of OSCC and observed that as grade of carcinoma progressed, the amount of collagen decreased.

Among fifty fields observed in 10 different sections stained with Picrosirius Red stain there was change in the colors of bundles of collagen fibers which varied from reddish orange in well differentiated OSCC to yellowish orange in moderately differentiated OSCC to greenish yellow in poorly differentiated OSCC. However, the normal mucosa stained section showed predominately reddish orange color.

Sharf et al [16] from their study also observed change in color profile from orange to red, which corresponds to the well packed fibers and green to greenish yellow to poorly packed fibers. The result of present study was also similar to finding of Kardam et al [12] and Kullage et al [17] who showed color variance of collagen fibers in different grades of OSCC.

According to van den Hooff [18] difference in birefringence of color in surrounding of tumor islands first could be due to action of enzymes secreted by tumor cells in immediate vicinity, secondly there would be abnormal disintegration of matrix by tumor cells, third the dedifferentiated tumor cells could be secreting abnormal matrix. Fourth, there could be formation of disorganized stroma around the tumor islands.

A subgroup of fibroblasts known as Cancer associated fibroblast (CAF) is thought to have a critical role in cancer initiation, progression, invasion and metastasis. The CAF in tumor stroma has a diverse origin. Majority are thought to arise from normal fibroblasts. The tumor cells induce epigenetic changes in normal fibroblasts and mutate them into CAF. They also rise when epithelial cells undergo epithelial-mesenchymal transition from trans-differentiated cells such as adipocytes, pericytes or smooth muscle cells. This mutated fibroblast phenotype contributes to the production of abnormal collagen. They also degrade the extracellular matrix protein by secretion of various growth factors, cytokines and chemokines, resulting in a disorganized abortive stroma.

In the initial grades, cancer invasion induces a desmoplastic reaction, i.e., formation of new extracellular matrix by activation of stromal cells. In progressive grades, the mechanical pressure exerted due to tumor growth and proteolysis induced by the tumor results in collagen degradation which facilitates tumor invasion.

The ECM molecules influence differentiation, proliferation & migration of epithelial tumor cells. The reactive alteration in the cancer stroma may modify the biological aggressiveness of oral cancer. The ECM has an effect on tumor behavior, improper synthesis or degradation of any ECM molecule can change cell functioning and thus helps in disease progression. Matrix metalloproteinase (MMPs) produced by cancer associated fibroblast as well as the inflammatory cells degrade the ECM structural components, both collagen and elastic and thus help in neoplastic progression.

Studies on stromal connective tissue in different grades of OSCC using special stains are few and more studies with a larger sample size are needed to be done, to confirm the connective tissue changes related to collagen fibers.

Conclusion:

The altered staining reactions of the collagenous stroma indicates their role in tumor invasion. This also leads to the assumption that tumor cells are capable of manipulating the ECM to enhance their own survival, which could be in the form of production and release of certain enzymes that alter or destroy the surrounding stroma. This paves a way for the need to develop newer therapies directed toward the modification of the stromal behavior thereby improving prognosis.

References:

[1]. Wright JM, Vered M. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Odontogenic and maxillofacial bone tumors. Head Neck Pathol. 2017; 11:68-77.

[2]. Uddin, N., Zubair, M., Abdul-Ghafar, J. et al. Orthokeratinized odontogenic cyst (OOC): Clinicopathological and radiological features of a series of 10 cases. Diagn Pathol **14,** 28 (2019).

[3]. Sabhalok SS, Shetty LS, Sarve PH, Setiya S V, Bharadwaj SR. Epidermoid and dermoid cysts of the head and neck region. Plast Aesthet Res 2016; 3:347-50.

[4]. Kasahara R, Tajiri R, Kobayashi K, Yao M, Kitami K. Squamous Cell Carcinoma Developing from a Testicular Epidermal Cyst: A Case Report and Literature Review. Case Rep Urol. 2019;2019:9014301.

[5]. 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.

[6]. New GB, Erich JB. Dermoid cysts of the head and neck. Surg Gynecol Obstet. 1937; (65):48.

[7]. Nigam JS, Bharti JN, Nair V, Gargade CB, Deshpande AH, Dey B, Singh A. Epidermal Cysts: A Clinicopathological Analysis with Emphasis on Unusual Findings. Int J Trichology. 2017 Jul-Sep;9(3):108-112.

[8]. Ma J, Jia G, Jia W. Primary intradiploic epidermoid cyst: A case report with literature review. Clin Neuropathol. 2019 Jan/Feb; 38(1):28-32.

[9]. Weir CB, St.Hilaire NJ. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Aug 11, 2021. Epidermal Inclusion Cyst.

[10]. Speight P, Fantasia JE, Neville BW. Orthokeratinized odontogenic cyst. In: El-Naggar AK, JKC C, Grandis JR, Takata T, Slootweg PJ, editors. WHO classification of head and neck tumors. 4th ed. Lyon: IARC; 2017. p. 241.

[11]. Patankar SR, Wankhedkar DP, Tripathi NS. Extracellular matrix in oral squamous cell carcinoma: Friend and Foe?. Indian J Dent Res 2016;27:184-9.

[12]. Kardam P, Mehendiratta M, Rehani S, Kumara M. Stromal fibres in oral squamous cell carcinoma: A possible new prognostic indicator?. J Oral Maxillofac Pathol 2016; 20:405-12.

[13]. MacDonald-Jankowski DS, Li TK. Orthokeratinized odontogenic cyst in a Hong Kong community: The clinical and radiological features. Dentomaxillofac Radiol. 2010; 39: 240–5.

[14]. Padmapriya VM, Kavitha B, Sivapathasundram B, Nagaraj J. Comparison of cytokeratin expressions among orthokeratinized odontoge nic cysts, epidermoid cysts and odontogenic keratocysts: An immunohistochemical study. J Oral Maxillofac Pathol 2020;24:472-8.

[15]. Khan S et al., A Histopathological Evaluation of Connective Tissue Stroma in Oral Squamous Cell Carcinoma: A Histochemical study. British Journal of Medical and Health Research 2020.

[16]. Sharf Y, Knubovets T, Dayan D, Hirshberg A, Akselrod S, Navon G. The source of the NMR detected motional anisotropy of water in blood vessel walls. Biophys J 1999;93:27-9.

[17]. Kullage S, Jose M, Shanbag VKL, Abdulla R. Qualitative analysis of connective tissue stroma in different grades of oral squamous cell carcinoma: A histochemical study. Indian J Dent Res 2017;28:355-61.

[18]. Van den Hoff A. Stromal involvement in malignant growth. Adv Cancer Res 1988;50:159-96.