ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 5, Issue – 2, March - 2022, Page No. : 376 - 387

Stromal changes in oral squamous cell carcinoma and field cancerization

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Citation of this Article: Dr. Divya Kanthi Kolli, Dr. A. Anuradha, Dr. G. Viaya Srinivas, Dr. K. Veda Priya, Dr. Bhavana Bagalad, Dr. Puneeth, "Stromal changes in oral squamous cell carcinoma and field cancerization", IJDSIR- March - 2022, Vol. – 5, Issue - 2, P. No. 376 – 387.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: The extracellular matrix is a dense latticework of collagen and elastin, embedded in a viscoelastic ground substance composed of proteoglycans and glycoproteins. It is a supporting scaffold which isolates tissue compartments, mediates cell attachment, and influences tissue architecture. The matrix acts as a selective macromolecular filter and plays a role in mitogenesis and differentiation. Interactions between normal cells and the matrix may be altered in neoplasia and this may influence tumor proliferation and invasion. Stromal changes play an active role in tumour progression.

Aim and objective: To study the stromal changes in lesion proper and adjacent mucosa by four different connective tissue stains and to unveil the link if any between tumour progression and connective tissue stroma.

Materials and method: The present study comprises of 30 samples of OSCC and 30 samples of tumour adjacent mucosa i.e., area of field cancerization. Sections of 5 micrometer thickness were taken serially on four slides and stained with four different connective tissue stains (i.e) PAS, alcian blue, aldehyde fuschin and picrosirius red. The slides were analysed using research microscope under 40X magnification. The data was recorded and analysed by un paired 't' test and chi square test.

Results: In PAS and alcian blue Intensity and pattern of distribution of stain were bright and uniform at papillary layer, reticular layer and submucosa of both tumour proper (TP) and tumour adjacent mucosa (TAM). Through picrosirius red immature haphazardly arranged

collagen fibers were seen in TP whereas mature parallel arrangement of collagen fibers was seen in TAM. Aldehyde fuschin staining showed haphazard arrangement of fibers in both TP & TAM whereas scanty fibers were seen in TP with moderate number of fibers at TAM.

Conclusion: An ambiguous ligation between connective tissue and tumour progression is essenced in this study.

Keywords: Oral Squamous Cell Carcinoma, Tumour proper, Tumour adjacent mucosa, Connective tissue, Stromal changes, Field Cancerization

Introduction

Oral cancer represents the third most common form of malignancy in the developing countries, whilst in the developed countries it is the eighth most common form of cancer, with common affected sites like tongue, lower lips and floor of the mouth[1]. In relation to OSCC, in 1953 Dr. Slaughter and colleagues introduced the term "field cancerization" or "field effect", which means occurrence of gentic and epigenetic alterations in histologically normal appearing tissues which is believed to be an increased risk of synchronous or metachronous primary tumors. Slaughter et al described the condition as "the presence of histologically abnormal tissue surrounding cancerous lesions". Field cancerization is an area of epithelium that has been pre conditioned by a yet unknown carcinogenic agent. Such carcinogenic influence if operative long enough in time and intense enough in exposure produces an irreversible change in the cell and cell groups in a given area, so that its progress towards cancer becomes inevitable[2].

Tumour is composed of two discrete interdependent components, the malignant cells themselves and the stroma in which they are dispersed. Presence of dysplastic areas in the epithelium is believed to be associated with a likely progression to cancer. Dysplastic features of a stratified squamous epithelium are characterized by cellular atypia and loss of normal maturation and stratification[3]. Although the epithelial component of oral SCC (OSCC) has been studied extensively, the stroma is still less considered. Differentiation, proliferation and migration of these epithelial tumor cells is influenced by extracellular matrix molecules and they also have stabilizing and separating functions.

Extracellular matrix is a network of collagen and elastin, embedded in a viscoelastic ground substance composed of proteoglycans and glycoproteins. Several types of fibrous proteins, including collagen, elastin, fibronectin and laminin are found in varying amounts within the extracellular matrix of different tissues[4].

The mature ECM consists of a supramolecular aggregate of connective tissue proteins, including fibrillar and non-fibrillar collagens, elastin, glycoproteins, glycosaminoglycans, acid mucins, neutral mucins, sulphated mucins, carboxylated mucins and fibrin The extracellular microenvironment of tumors is determined by matrix synthesized by normal and tumor cells, as well as the host stromal components secreted by surrounding 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[4]. In addition to carcinoma cells, various cellular and acellular stromal components contribute to promoting and maintaining HNSCC invasion. This tumour stroma can be identified and studied by using various histochemical stains like periodic acid Schiff stain (PAS), Alcianblue, Aldehydefuschin, Picrosirious red

PAS stain is used for the demonstration of glycogen. Tissue sections are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groupings through carbon-to-carbon bond cleavage. The

aldehyde groups are detected by the Schiff reagent. A colorless, unstable di aldehyde compound is formed and then transformed to the magenta-colored final product. Alcian blue staining is widely used to visualize mucopolysaccharides and acidic mucins. Aldehyde fuschin stain demonstrates carboxylated mucins and elastic fibers present in tumour stroma. Picrosirius red stains collagen fibers. Polarizing colours of the collagen fibers were demonstrated as a gradual Change from reddish orange to greenish yellow from well to poorly differentiated squamous cell carcinoma, indicating the tumour progression and a change from the mature form of collagen to an immature form.

Materials and Methods

The present study was carried out in the department of Oral and Maxillofacial Pathology, St. Joseph Dental College and Hospital, Eluru. The study samples were collected from patients attending the outpatient department of Good Samaritan cancer hospital, Vangayagudem, Mahatma Gandhi cancer hospital, Visakhapatnam and St. Joseph Dental College and Hospital, Eluru. A total of 30 subjects who were diagnosed with OSCC were included under the study group. The study group was divided into 2, in Group 1 Biopsy taken from tumour proper, in Group 2 Biopsy taken from tumour associated mucosa (i.e) 1 cm away from lesion boundary.

Diagnosed oral squamous cell carcinoma patients in whom treatment is not initiated are included and Patients with any other systemic diseases, debilitated patients, cachexic patients, patients with recurrent tumours are excluded.

Biopsies were processed and Sectioning of paraffin blocks using microtome was performed. 5 microns thick sections were mounted on microscopic slides. Staining was done using PAS stain, Alcian blue, Aldehyde fuschin, Picrosirous red as per the prescribed protocol and evaluation was done. This evaluation of the slide was based on the following criteria: intensity of stain, Pattern of distribution, maturity of fibers, arrangement of fibers and density of fibers.

Observation

1. Periodic acid Schiff stain (PAS) stains Glycogen and various Glycoproteins. Mucocele was taken as control tissue. Glycogen and various glycoproteins will stain Magenta and nuclei in Blue colour.
2. Alcian Blue stains acid mucins and control tissue as salivary gland. These acid mucins will stain in Blue colour: Proteoglycans and Hyaluronic acid stains Blue and nuclei in Red.
3. Picrosirus Red stains collagen fibers and control was Oral Sub mucous Fibrosis. Mature and immature collagen fibers appear as reddish orange to greenish yellow.
4. Aldehyde fuschin stains elastic fibers and control tissue was Salivary gland. This stain used for staining of sulfated mucins and elastic fibers which appear in blue colour and carboxylated mucins in purple colour.

Results

For PAS staining considering the intensity of staining of stroma of tumour proper and tumour adjacent mucosa, mean staining intensity of tumour proper was more when compared to the staining intensity of tumour adjacent mucosa with mean difference of 0.1 but statistically insignificant because the p value was 0.3 which is >0.05. Thus staining intensity of both tumour proper and tumour adjacent mucosa are same. When pattern of distribution of stain in papillary layer considered, there is no significant difference in pattern of distribution of stain between tumour proper and tumour adjacent mucosa (P>0.05). 80% of the tumour proper showed uniform distribution of stain with only 20% displaying

patchy areas whereas 93.33% of Tumour adjacent mucosa showed uniform distribution. Thus both TAM and TP stained uniformly with PAS. Then pattern of distribution of stain in reticular layer considered there is no significant difference in pattern of distribution of stain between tumour proper and tumour adjacent mucosa (P>0.05). 73.33% of the tumour proper showed uniform distribution of stain with only 26.67% displaying patchy areas whereas 80% of Tumour adjacent mucosa showed uniform distribution. Thus, both TAM and TP stained uniformly with PAS. Coming to pattern of distribution of stain in sub mucosa, there is significant difference in pattern of distribution of stain in both tumour proper and tumour adjacent mucosa (P>0.05).33.33% of the tumour proper showed uniform distribution of stain with 66.67% displaying patchy areas whereas 66.67% of Tumour adjacent mucosa showed uniform distribution. Thus uniformity is more in tumour adjacent mucosa, patchy areas are more in tumour proper.

For Alcian blue staining considering the intensity of staining of stroma of tumour proper and tumour adjacent mucosa, mean staining intensity of tumour proper was equal to staining intensity of tumour adjacent mucosa with 0 mean differences and statistically insignificant because the p value was 0.9 which is >0.05. Thus staining intensity of both tumour proper and tumour adjacent mucosa were same. Considering the pattern of distribution of stain in papillary layer, there was no significant difference in pattern of distribution of stain between tumour proper and tumour adjacent mucosa (P>0.05). 76.67% of the tumour proper showed uniform distribution of stain with only 23.33% displayed patchy areas whereas 83.33% of Tumour adjacent mucosa showed uniform distribution. Thus both TAM and TP stained uniformly with Alcian blue. Coming to pattern of distribution of stain in reticular layer, there was no significant difference in pattern of distribution of stain between tumour proper and tumour adjacent mucosa (P>0.05). 66.67% of the tumour proper showed uniform distribution of stain, 33.33% displayed patchy areas, whereas 80% of Tumour adjacent mucosa showed uniform distribution. Thus both TAM and TP stained uniformly with Alcian blue. Then pattern of distribution of stain in sub mucosa considered, there was no significant difference in pattern of distribution of stain between tumour proper and tumour adjacent mucosa (P>0.05). 63.33% of the tumour proper showed uniform distribution of stain with only 36.67% displayed patchy areas whereas 76.67% of Tumour adjacent mucosa showed uniform distribution. Thus both TAM and TP stained uniformly with Alcian blue.

For picrosirius red staining, when intensity of staining of stroma of tumour proper and tumour adjacent mucosa considered, mean value of staining intensity of tumour proper was marginally more when compared to staining intensity of tumour adjacent mucosa, p value was 0.3 which was statistically insignificant. Coming to maturity of fibers, there was no significant difference in maturity of fibers between tumour proper and tumour adjacent mucosa (P>0.05). 56.67% of the tumour proper showed immature fibers 43.33% were mature fibers whereas 33.33% of Tumour adjacent mucosa showed immature fibers and 66.67% were mature fibers. Thus TAM showed more mature and TP showed more immature fibers with picrosirius red. Considering the arrangement of fibers, there is no significant difference in arrangement of fibers between tumour proper and tumour adjacent mucosa (P>0.05). 53.33% of the tumour proper showed haphazard arrangement of fibers with only 46.67% displaying parallel arrangement to tumour island whereas 30% of Tumour adjacent mucosa showed

haphazard arrangement and 70% showed parallel arrangement but statistically insignificant. Thus, TAM showed parallel arrangement and TP showed more haphazard arrangement of fibers with picrosirius red.

For Aldehyde fuschin staining when intensity of staining of stroma of tumour proper and tumour adjacent mucosa considered, mean value of staining intensity of tumour proper is was greater when compared to staining intensity of tumour adjacent mucosa, with mean difference of 0.03, P value was 0.7 which was statistically insignificant. When density of fibers considered, there is significant difference in density of fibers between tumour proper and tumour adjacent mucosa. (P>0.05).73.33% of the tumour proper showed scanty amount of fibers with only 26.67% displaying moderate availability. Whereas 53.33% of Tumour adjacent mucosa showed moderate availability and 46.67% showed scantiness. Thus TAM showed moderate presence and TP showed scanty presence of elastic fibers with aldehyde fuschin. Then coming to arrangement of fibers, there is no significant difference in arrangement of fibers between tumour proper and tumour adjacent mucosa(P>0.05).70% of the tumour proper showed haphazard arrangement of fibers with only 30% displaying parallel arrangement to tumour island whereas 83.33% of Tumour adjacent mucosa showed haphazard arrangement and 16.67% showed parallel arrangement. Thus both TAM and TP showed more haphazard arrangement of fibers with aldehyde fuschin.

Discussion

Squamous cell carcinoma (SCC) consists of two interdependent components – the tumor epithelial cells and the stroma. These components interact on a regular basis and to some extent discrepancies in one could affect the other. Hence, for a better prediction of course and outcome of SCC, both should be given equal weightage during histologic evaluation. Epithelium has been studied extensively by various authors considering the dysplastic features and tumour invasive front contributing to the progression of tumour.[5] Compared to the tumour epithelium, tumor stroma is studied sporadically even today. Among the stromal components, only the inflammatory component has been included in few of the SCC grading systems. The other stromal constituents, i.e., the glycoproteins, acid mucins, neutral mucins, collagen, elastic fibers and several other extracellular molecules are still in store for future research. According to George et al stroma is not just a passive structure but is associated with tumor progression. Endothelial cells, pericytes, inflammatory cells, fibroblasts and extracellular matrix constitute the tumor milieu or tumor microenvironment which helps in tumor growth, invasion and metastasis.[6, 7, 8]

Along with the tumour proper, the epithelium around tumour, which is clinically normal endowed some histological changes. The whole mucosa having exposed to the carcinogenic agent develops susceptibility in developing more than one independent foci of malignant transformation. This clinically apparent normal mucosa is called field cancerization. The term **"**field cancerization**"** or "field effect" was originally introduced by Dr. Slaughter and colleagues in 1953 related to oral squamous cell carcinoma. Slaughter et.al described the condition as "the presence of histologically abnormal tissue surrounding the cancerous lesions"[9,10]. In the present study stromal changes in both tumour proper and tumour adjacent apparently normal mucosa are studied parallely to reveal the role of connective tissue stroma in tumour initiation and tumour progression[11, 12].

The tumour progression is accompanied by degradation of the basement membrane and components of matrix

which occur at several stages of metastatic cascade, including local invasion, angiogenesis, vascular and lymphatic invasion[13,14]. Connective tissue was divided into 3 different layers papillary layer, reticular layer and submucosa in this study. Various components like glycoproteins, acid mucins, neutral mucins, sulphated mucins, elastic and collagen fibers were studied by using different connective tissue stains such as PAS, Alcian blue, Aldehyde fuschin and Picrosirius red.

PAS stain is used for demonstration of glycoproteins and neutral mucins. In this study staining characteristic of PAS were evaluated on the basis of pattern of distribution and intensity. Considering the pattern of distribution of stain, in papillary layer, reticular layer and submucosa in both TP and TAM the stain was uniformly distributed which is equable to the distribution of ground substance that contains glycoproteins, mucins, glycosaminoglycans uniformly. Few areas of patchy distribution in TP may be due to regressive changes such as the lysis of stromal components, creating pathway for cell migration or change in stromal protein composition which may be induced by mediators derived from tumor cells.

Intensity of PAS staining was more around the tumour islands/nests which could probably represent basement membrane components secreted by the tumor cells. Basement membrane contains glycoproteins, glycosaminoglycans, type IV collagen, laminin, entactin etc. Many studies have proposed that malignant epithelial cells continue to synthesize, secrete and assemble basement membrane (BM) materials, such as laminin, entactin and heparan sulphate[15,16]. Fibroblasts and macrophages of the stroma express and secrete MMPs which is the result of complex tumor-stroma crosstalk, involving multiple ligands and cellular signaling pathways. As a family of compounds, the MMPs act to hydrolyze the extracellular proteins of the surrounding tissue which include collagen, laminin, elastin, fibrinogen and fibronectin. MMPs have also been implicated in initiating the EMT and in promoting genomic instability, affording them a prominent role in both tumor progression and prevention[17,18].

Alcian blue is used for staining of acid mucins and sulphoproteins. When compared to PAS, intensity of staining was less due to the weak presence of acid mucins when compared to neutral mucins. Papillary layer, reticular layer and submucosa of both TP and TAM showed uniform distribution of stain due to coequal presence of acid mucins and sulphoproteins in the stroma. In TP bright intensity of stain was observed where as in TAM moderate staining intensity was observed but the difference was statistically insignificant $p>0.05$. This variation could be subjective as borders were not drawn to measure the level of intensity.

By using birefringence pattern of Picrosirius red stain (PSR) the ratio of thick to thin fibers and pattern of arrangement of collagen fibers around tumour islands was determined using polarizing microscopy. The mechanical quality of ECM is mainly dependent on its collagenous content and it is the presence of collagen which is considered a main barrier to be cleared away during invasion, thus making room for infiltrating cell mass. MMPs are a group of proteolytic enzymes which degrade most of the components of ECM. The MMP system consists of 23 MMPs which are further divided into five groups, namely, gelatinase, collagenase, Strome lysins, membrane type MMPs and less well characterized MMPs. Type I collagen which is about 90% and type III collagen which is 8–10% constitute major components extracellular matrix. Electron microscopic studies have shown that type I collagen fibers are coarse and are composed of closely packed

thick fibrils, whereas type III collagen are thin fibers and they are loosely disposed[19]. In the present study, intensity of staining of picrosirius red was same in both tumour proper and tumour adjacent mucosa due to uniform distribution of collagen fibers.

When Maturity of fibers compared, was accounted out of 30 slides of tumour proper, 13 showed mature fibers and 17 showed immature fibers with colours ranging from yellowish red to greenish yellow. Where as in tumour adjacent mucosa, out of 30, 20 showed mature fibers and 10 cases showed immature fibers with colour ranging from yellowish red to greenish yellow. But this difference was statistically insignificant. Predominance of these immature fibers and marked colour changes were more around the tumour islands. Presence of these immature fibers may be due to lysis of stroma which was an essential requirement for invasive growth. It is seen that collagen disintegrates that is they undergo “elastotic degeneration”. Electron micrographic studies have shown diffuse collagenolysis and phagocytosis of intact collagen fibrils in the course of carcinomatous transformation. Malignant epithelial cells produce various lytic enzymes like cathepsin, elastolytic and glycosaminoglycan degrading enzymes which attack the stroma and induce the fibroblast to synthesize collagenolytic activities resulting in immature fibers appearing as greenish yellow[20]. The reason for this color change occurring predominantly in the immediate vicinity of tumor islands is still not clear. vanden Hooff et al[21] suggested that this difference in birefringence of color surrounding the tumor islands could be firstly due to the action of enzymes such as collagenases or the metalloproteinases secreted by tumor cells on the collagen in the immediate vicinity. Additionally, there could also be an abnormal disintegration of the matrix by the tumor cells. More over the dedifferentiated tumor cells could be secreting an abnormal matrix. Finally there could be a formation of disorganized or abortive stroma around the tumor islands.

The physical aggregation of collagen which contributes to its birefringence could be altered due to action of collagenases, matrix metallo proteinases (MMPs) secreted by stromal cells. Thus in this study, the presence of immature fibers in stroma of TAM along with TP, reveals the stromal changes contributing to the progression of carcinoma. Similar to our study, Arun Gopinath an et al[22, 23, 24] observed an identical pattern of color change of thick collagen fibers in different grades of oral squamous cell carcinoma which was found to be statistically significant. It is known that the birefringence color of a collagen fiber depends on its diameter and packing.

The decrease in thick fibers and increase in thin fibers as the grade of carcinoma is increasing can be explained by the fact that during the initial stage there would be a fibroproliferative response and in later stages there will be abnormal collagen production and defective maturation which may foster thin fiber formation and neoplastic growth. Studies on upper respiratory tract neoplasms have also shown that total collagen volume decreases with increasing degree of malignancy from well differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC) to poorly differentiated squamous cell carcinoma (PDSCC) and also collagen fiber size decreases in less differentiated SCC. The pattern of arrangement of fibers was categorized into parallel and haphazard. Almost equal number of cases showed haphazard and parallel arrangement in tumour proper whereas majority of cases showed parallel arrangement of fibers at Tumour adjacent mucosa. As the grade of OSCC progressed, the packing of collagen fibers

decreased, and the orientation of collagen fibers changed from parallel to haphazard. This could possibly be due to some enzymatic degeneration of the existing collagen or due to the formation of new abnormal/pathologic collagen. Moreover, it could also be the result of increased amount of Type III collagen which occurs singly rather than in bundles like Type I collagen. Thus in TAM or FC the stroma does not show much of disorganization in orientation of fibers.

Ziober et al[25] in their study examined the production of matrix metalloproteinases (MMPs) in the invasive OSCC and observed that MMP 1 cause degradation of type I collagen, thus indicating a decrease in collagen content and arrangement of collagen fibers during invasion. Moreover, George et al. in their study evaluated the response of stroma in varying grades of OSCC and observed that as the grade of carcinoma progressed, the amount of collagen decreased. Similarly, Martin et al in their study on OSCC observed that in a lower grade of malignancy (i.e., well differentiated OSCC) collagen fibers near invasive front were arranged parallel to the tumor islands. However, in higher grades of malignancy, the ECM exhibited lower levels of collagen synthesis and the fibers were irregular, disorganized and dissociated[26]

The intensity of staining of Aldehyde fuschin in tumour proper and tumour adjacent mucosa was same due to the presence of proportionate amount of elastic fibers in both. Both groups showed haphazard fiber arrangement primly with meger cases demonstrating parallel pattern. These findings are in accordance with kardam et al[5,27] who demonstrated that the elastic fibers are predominantly haphazardly arranged in well and moderately differentiated OSCCs. However in poorly differentiated OSCCs parallel orientation was predominantly seen.

The density of fibers in TP was scant in majority of cases where as in TAM 16 cases showed moderate density whereas 14 showed scanty elastic fibers. This scantiness is in accordance with study conducted by Zhang et al[28] which revealed a decrease in elastic fibers with progression from epithelial atypia to early invasive carcinoma. There is an interaction between the tumor cells and elastic fibers, but its effect on the progression of carcinoma is questionable. Tux horn et al[29, 30, 31] quoted that the cancer cells can interact specifically with elastin through two elastin binding proteins and galectin 3. Lapis and Timar have suggested that there is a positive correlation between tumor progression and the presence of elastic fibers in the tumor stroma. The sparse literature, lesser number of elastic fibers in lamina propria and the masking effect of overlying inflammatory cells could be the limiting factors in the assessment of elastic fibers in OSCC. These results of the present study elucidate the role of tumour cells in manipulating the ECM for better survival, migration and progression of cancer.

Conclusion

Present study was an attempt to unveil the link between tumour progression and connective tissue stroma. The results of present study showed uniform distribution of stain at papillary layer, reticular layer and sub mucosa with PAS and Alcian blue in both TP and TAM. Intensity of stain was undiversified in all four stains in both TP and TAM.

Immature and haphazard arrangement of collagen fibers at TP and mature and parallel arrangement at TAM were observed by picrosirius red stain. Scanty and haphazard arrangement of elastic fibers at TP and moderate and haphazard arrangement of fibers at TAM were observed with aldehyde fuschin.

Thus the presence of coequal distribution of stain and arrangement of fibers at both areas revealed that the stroma plays a major role for tumour initiation and tumour progression. Along with tumour proper, tumour adjacent mucosa showed similar stromal changes contributing the formation of second primary tumours.

References

1. Doshi Neena, Shah Siddharth, Patel Keyuri, Jhabuawala Munira. Histological grading of oral cancer: a comparison of different systems and their relation to lymph node metastasis. National journal of community medicine 2011; 2 (1):136-42.
2. Meenakshi Mohan, Nithya Jagannathan. Oral field cancerization: an update on current concepts Oncology Reviews 2014; 8(244):13-17
3. S. Warnakulasuriya, J. Reibel, J. Bouquot, E. Dabelsteen. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med 2008; 37: 127–133
4. 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(6): 842
5. Kardam P, Mehendiratta M, Rehani S, Kumra M, Sahay K, Jain K. Stromal fibers in oral squamous cell carcinoma: A possible new prognostic indicator? J Oral Maxillofac Pathol 2016;20: 405-12.
6. Hanchen Li, Xueli Fan, and Jean Marie Houghton Tumor Micro environment: The Role of the Tumor Stroma in Cancer Journal of Cellular Biochemistry 2007.101:805–15
7. Lin Ge, Wenxia Meng, Hongmei Zhou, Neil Bhowmick Could stroma contribute to field cancerization? Medical Hypotheses 2010;75: 26–31.
8. G. Paolo Dotto. Multifocal epithelial tumors and field cancerization: stroma as a primary determinant. J Clin Invest 2014;124(4):1446–1453.
9. Aparna M, Shenai P, Chatra L, Veena KM, Rao PK, Prabhu RV, et al. Field cancerization: VA review. Arch Med Health Sci 2013; 1:136-9.
10. Slaughter D. P., Southwick H. W., Smejkal W. Field cancerization in oral stratified squamous epithelium. Cancer (Phila.) 1953; 6: 963968.
11. Sharma M, Sah P, Sharma SS, Radhakrishnan R. Molecular changes in invasive front of oral cancer. J Oral Maxillofac Pathol 2013; 17:240-7
12. Pengfei Lu, Valerie M. Weaver, and Zena Werb The extracellular matrix: A dynamic niche in cancer progression J. Cell Biol. Vol. 2012;196(4):395–406
13. Agrawal U, Rai H, jain A K. Morphological and Ultrastructural chracteristics of extracellular matrix changes in oral squamous cell carcinoma. Indian journal of dental research.2011;22(1):16-21.
14. Sgarbi FC, Bertini F, Tera TDM, Cavalcante ASR. Morphology of collagen fibers and elastic system fibers in actinic cheilitis. Indian J Dent Res 2010; 21:518-22.
15. Isaac P. Witz The Tumor Microenvironment: The Making of a Paradigm Cancer Microenvironment 2009; 2(1):9-17.
16. Frances R. Balkwill, Melania Capasso and Thorsten Hagemann. The tumor microenvironment at a glance Journal of Cell Science 2012;125(3): 5591–5596
17. Lance A.Liotta Tumor Invasion and metastasis. Role of the Extracellular Matrix: Rhoads Memorial Award Lecture. Cancer research 1986; 46:1-7.
18. Sittichai Koontongkaew. The Tumor Microenvironment Contribution to Development, Growth, Invasion and Metastasis of Head and Neck Squamous Cell Carcinomas. Journal of Cancer 2013; 4(1): 66-83.

19. Raed Lattouf, Ronald Younes, Didier Lutomski, Nada Naaman, Gaston Godeau, Karim Senni, and Sylvie Changotade Picrosirius Red Staining: A Useful Tool to Appraise Collagen Networks in Normal and Pathological Tissues Journal of Histochemistry & Cytochemistry 2014, Vol. 62(10) 751– 58

20. Rashi Sharma, Shweta Rehani, Monica Mehendiratta, Priyanka Kardam, Madhu Mani Kumra, Yulia Mathias, Jyoti Yadav, Khushboo Sahay. Architectural Analysis of Picrosirius Red Stained Collagen in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma using Polarization Microscopy. Journal of Clinical and Diagnostic Research 2015; 9(12):13- 16.

21. Pillai Arun Gopinath an, Gang anna Kokila, Mahadesh Jyothi, Chatterjee Ananjan, Linganna Pradeep, Salroo Humaira Nazir. Study of Collagen Birefringence in Different Grades of Oral Squamous Cell Carcinoma Using Picrosirius Red and Polarized Light Microscopy. Scientific Volume 2015, 7 pages

22. A. van den Hooff, Stromal involvement in malignant growth, Advances in Cancer Research.1988; 50:159–196

23. John RE, Murthy S. Morphological analysis of collagen and elastic fibers in oral squamous cell carcinoma using special stains and comparison with Broder's and Bryne's grading systems. Indian J Dent Res 2016; 27:242-8.

24. 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.

25. B. L. Ziober, M. A. Turner, J. M. Palefsky, M. J. Banda, and R. H. Kramer. Type I collagen degradation by invasive oral squamous cell carcinoma. Oral Oncology.2000; 36(4):365–372

26. Sittichai Koontongkaew. The Tumor Microenvironment Contribution to Development, Growth, Invasion and Metastasis of Head and Neck Squamous Cell Carcinomas. Journal of Cancer 2013; 4(1): 66-83

27. Patricia SL, Suzigan S, Carvalho HF, Taboga SR. Structural characterization and distribution of elastic system fibers in the human prostate and some prostatic lesions. Braz J Morphol Sci 2003; 20:101-7.

28. Zhang P, Du YB, Yu M, Yin X, Lv YH, Gao ZX, et al. Changes of the elastic fibers and collagen fibers during the development and progression of experimentally induced tongue carcinoma in hamsters. Nan Fang Yi Ke Da Xue Xue Bao 2010;30: 2696-8

29. Tux horn JA, Ayala GE, Rowley DR. Reactive stroma in prostate cancer progression. J Urol 2001;166 :2472- 83.

30. Lapis K, Tímár J. Role of elastin-matrix interactions in tumor progression. Semin Cancer Biol 2002; 12:209-17

31. Patricia SL, Suzigan S, Carvalho HF, Taboga SR. Structural characterization and distribution of elastic system fibers in the human prostate and some prostatic lesions. Braz J Morphol Sci 2003; 20:101-7.

Legend Figures

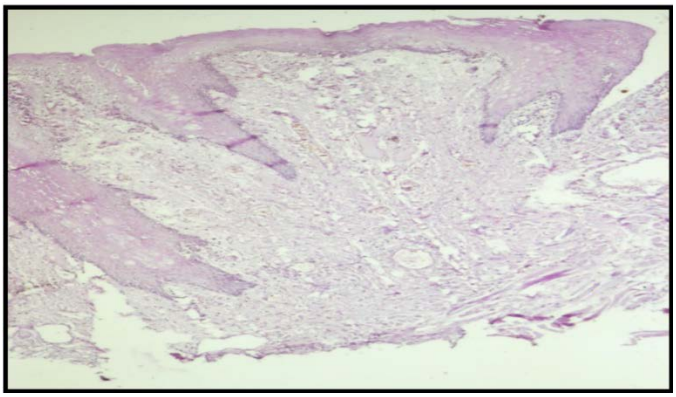

Figure 1: Uniform Pattern of distribution of PAS stain in tumor adjacent mucosa.

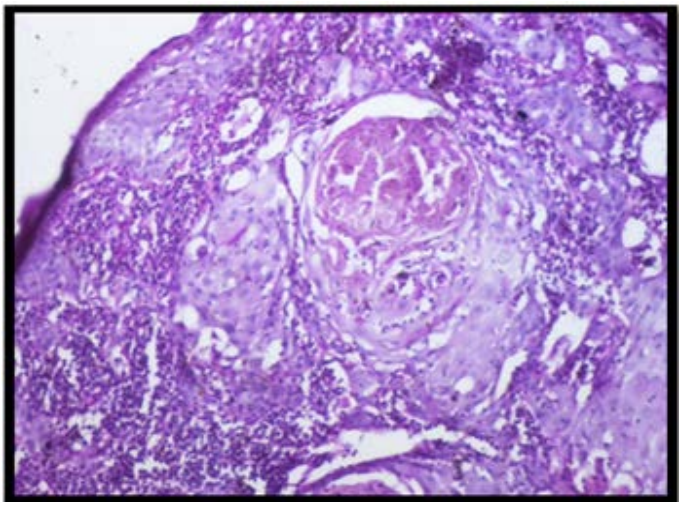

Figure 2: Uniform Pattern of distribution of PAS stain in tumor proper.

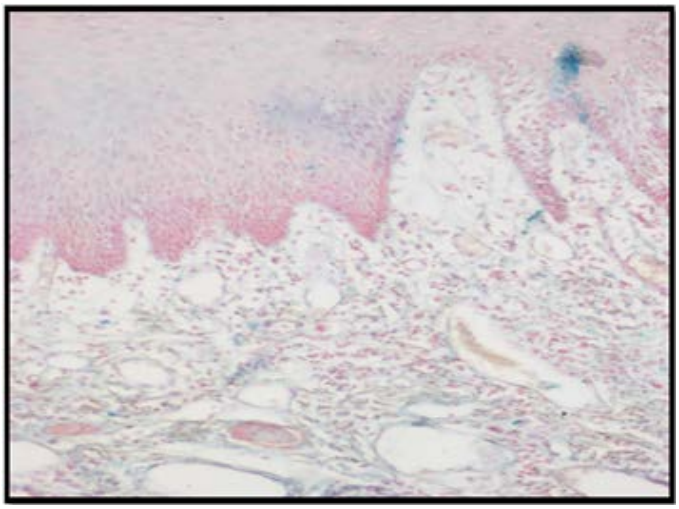

Figure 3: Uniform Pattern of distribution of alcian blue stain in tumor adjacent mucosa.

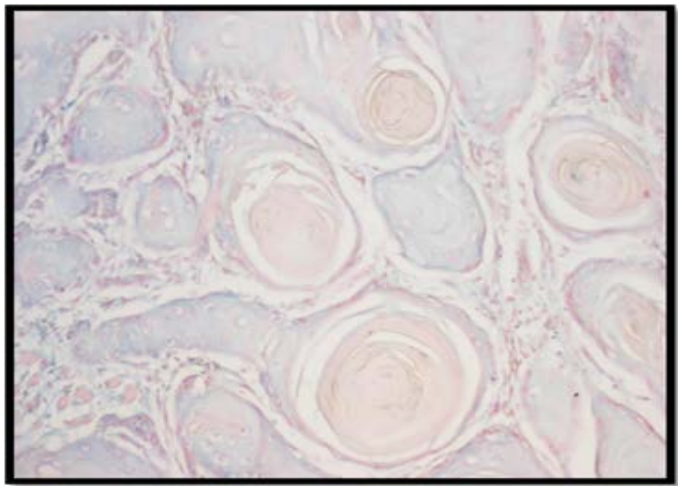

Figure 4: Uniform Pattern of distribution of alcian blue stain in tumor proper.

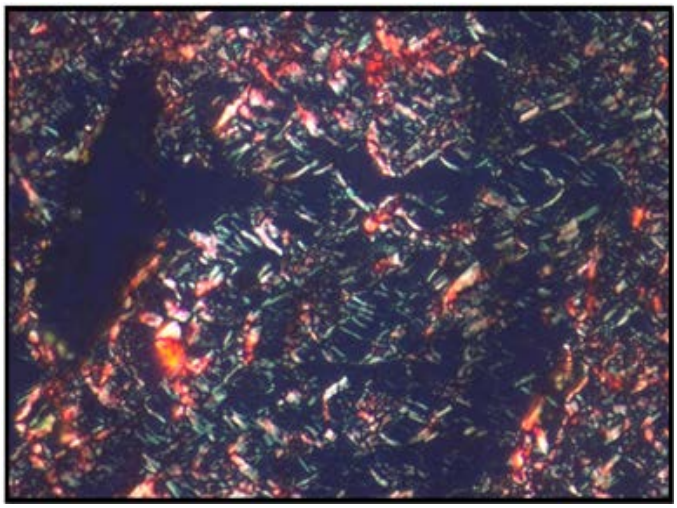

Figure 5: Mature and parallelly arranged collagen fibers stained with PSR in tumor adjacent mucosa.

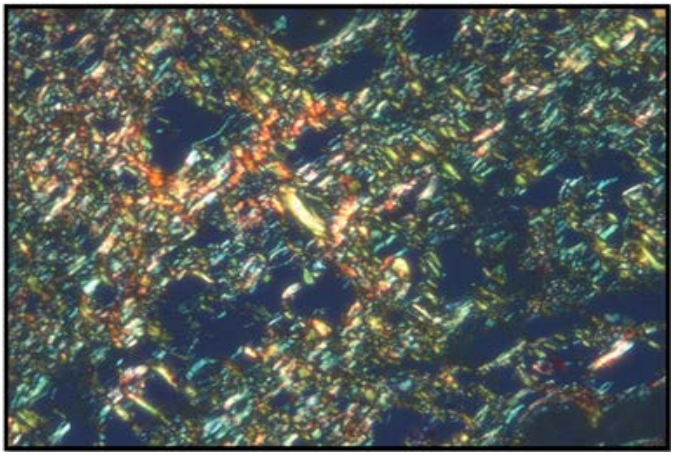

Figure 6: Immature and haphazardly arranged collagen fibers with PSR in tumor proper.

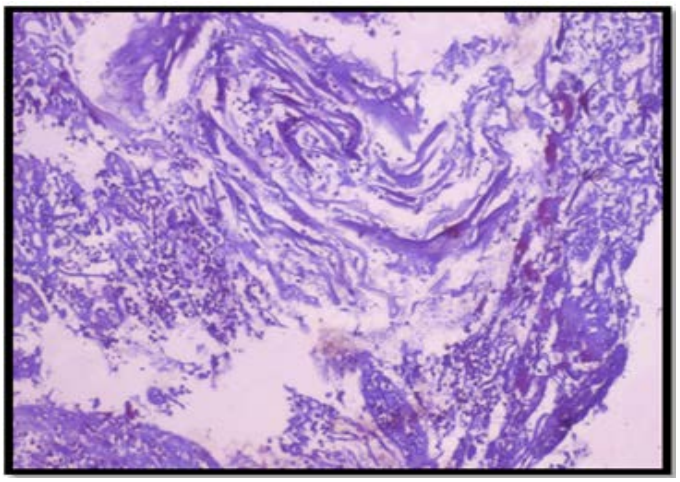

Figure 7: Moderate and haphazardly arranged elastic fibers stained with aldehyde fuschin in tumor adjacent mucosa.

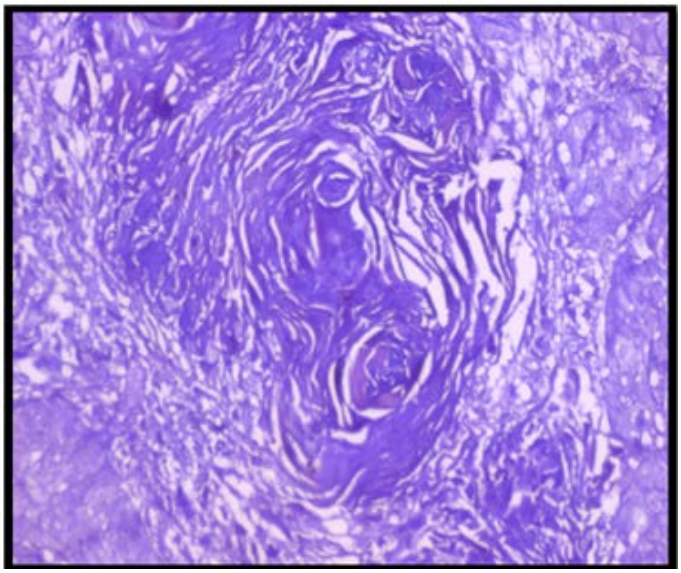

Figure 8: Scant and haphazardly arranged elastic fibers stained with aldehyde fuschin in tumor proper.