Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
PubMed (National Library of Medicine): ID: (101671502)
Research Article
Volume 8, Issue 4: July-August: 2019, 48-55
ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

COMPARISON OF IMMUNOHISTOCHEMICAL MARKER WITH SPECIAL STAINS IN THE PROGNOSIS OF THE DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA

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Article Info: Received 15 July 2019; Accepted 30 August. 2019
DOI: https://doi.org/10.32553/jbpr.v8i4.640
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Conflict of interest statement: No conflict of interest

ABSTRACT:

Objectives: The aim of the study is to compare the efficiency of Ki-67 with special stains in the stroma of different grades of Oral Squamous Cell Carcinoma (OSCC) and to evaluate the influence of these changes in predicting the prognosis of these tumors.

Materials and Methods: A total of 30 cases of different grades of Oral Squamous Cell Carcinoma and 6 cases of control were sections and stained with Picrosirus red (PR), combined Alcian blue-periodic acid Schiff reagent (AB-PAS) and Immunohistochemical (IHC) marker Ki-67.

Results: Collagen fibre nature using PR stain and proliferative activity of malignant epithelial cells using IHC marker Ki-67 was found to be statistically significant. Mucin presence using AB-PAS was statistically insignificant.

Conclusion: Prognosis in different grades of oral squamous cell carcinoma can be accessed by change in collagen fibres birefringence, as the tumour progresses there is change from mature to immature collagen. Ki-67 is a good proliferative marker and shows that there is positive correlation with histological grading of oral squamous cell carcinoma. Presence of acidic or neutral mucin in OSCC needed to be further studied. For assessing the prognosis in different grades of OSCC, special stains can also be used.

Keywords: Collagen, Mucin, Squamous cell carcinoma, Ki-67.

INTRODUCTION

The oral cavity is the site where food is received and therefore an area of body where contact with exogenous material, microorganism and harmful agents is particularly intense. The oral mucosa functions as a mechanical as well as immunological barrier.[1] Oral cancer holds the eighth position in the cancer incidence ranking worldwide, with epidemiologic variations between different geographic regions (it is the third most common malignancy in south-central Asia).[2]

Oral cancer includes a group of neoplasms affecting any region of the oral cavity, pharyngeal regions and salivary glands.[3] The most common malignant epithelial neoplasm in the oral cavity is the oral squamous cell carcinoma (OSCC), representing over 90% of malignancies of the oral cavity.[4] Oral Squamous cell carcinoma is a malignant neoplasm derived from the stratified squamous epithelium of the oral mucosa.[5] Histopathological grading is an important factor in determining the prognosis of oral carcinoma.[6]

The Ki-67 is a nuclear non-histone protein, encoded by a gene located on chromosome 10q25-ter, containing phosphorylation sites for a variety of kinases, playing roles in cell cycle regulation, synthesis of ribosomes and also being related to

survival, malignancy and prognosis of various neoplasms, including OSCC. Ki-67 is an easy and useful method because it can provide useful information for the prognosis of the lesions. In OSCC, the increased rate of Ki-67 has been correlated with poor survival, high degree of malignancy and histological grading in the invasive front.[4]

Tumour progression in OSCC is associated with a reorganization of extracellular matrix (ECM).[7] The ECM is composed of fibrous components such as collagen, elastin and reticulin fibers and ground substance which include glycoproteins, mucins and fibrin.[8]

Collagens play an important role in OSCC development and progression, although the mechanisms remain unknown.[9] The collagen around the tumor cell nests shows thick fibrous bundles arranged parallel to the epithelium.[10] Examination of collagen fibres by Picriosirus red under Polarizing microscopy shows, a gradual change from reddish orange to greenish yellow from well to poorly differentiated SCC. These changes in the collagen fibres in stroma aids in predicting tumour behaviour.[11]

Mucins are large extracellular proteins that are heavily glycosylated with complex oligosaccharides, which establish a selective molecular barrier at the epithelial surface and engage in morphogenetic signal transduction.[12] Tumours might use mucins to control the local microenvironment during inappropriate invasive and metastatic growth in different organ and tissue sites, which could allow them to survive and proliferate.[12] Combined Alcian blue- Periodic Schiff reagent (AB-PAS) staining procedure will stain all different types of mucins where the Neutral mucin is in Magenta colour, Carboxylated mucin in Blue colour and Sulphated mucin in Purple colour.[13] Transmembrane mucins are overexpressed in malignant cells, which have been useful in cancer diagnosis and prognosis.[14]

With the above facts, the main purpose of this study is to compare the nature of collagen fibres and presence of acidic and neutral mucin in different grades of oral squamous cell carcinoma, with the proliferative activity of malignant epithelial cells in the stroma using Immunohistochemical marker Ki-67 and to analyze whether these factors can be useful in predicting the prognosis of oral squamous cell carcinoma.

AIM AND OBJECTIVES

To analyze the expression of Immunohistochemical marker Ki-67, evaluation of collagen fibres and presence of acidic or neutral Mucin in the stroma of different grades of the Oral squamous cell carcinoma.

To compare the collagen fibres and presence of acidic or neutral mucin in the stroma using Picrosirus red and combined Alcian blue – Periodic acid Schiff stain respectively and the Immunohistochemical marker Ki-67 in the Well differentiated, Moderately differentiated and Poorly differentiated Oral Squamous cell carcinoma

To determine whether these changes in collagen and mucin can be useful in predicting the prognosis of the Well differentiated, moderately differentiated and Poorly differentiated squamous cell carcinoma.

MATERIALS AND METHODS

The study was conducted by retrieving the paraffin blocks from the archives of Department of Oral Pathology and Microbiology, Tamil Nadu government Dental college & Hospital, Chennai. 4 sections of 3 microns were cut from each block and each group was stained with Haematoxylin & Eosin, Alcian blue Periodic acid Schiff stain (AB-PAS), Picrosirus red (PR) and Immunohistochemical marker Ki-67. Sections of 3 microns were taken in an albumin coated slides for H&E, AB-PAS stain and Picrosirus red stain. For Immunohistochemistry (IHC), sections were taken in aminopropyltriethoxysilane (APES) coated slides. Scoring was done by the Principal Investigator and Co-investigator.

Scoring criteria for Ki-67

Strong brown nuclear staining of epithelial cell was considered positive. The section stained for Ki-67 proliferation were evaluated using scores 1 to 3.

1. High proliferation - > 60% positive cells.
2. Moderate proliferation – between30% to 60% of positive cells
3. Low proliferation – < 30% of positive cells

Scoring criteria for Mucin

The section stained with AB-PAS stain were evaluated using scores 1 to 3.

1. Presence of Acidic mucin > 50%
2. Presence of Neutral mucin > 50%
3. Presence of both Acidic and Neutral mucins.

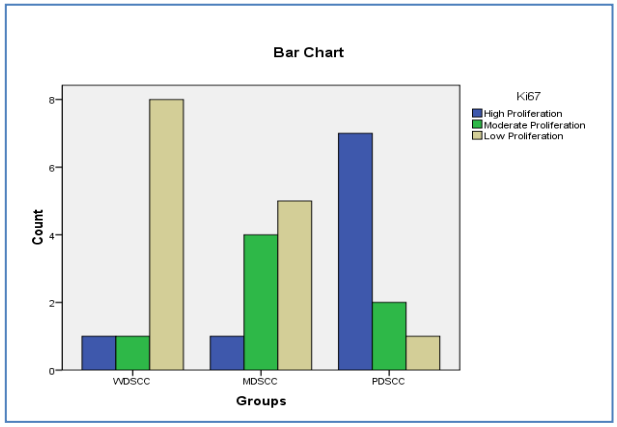
Scoring criteria for collagen fibres

The section stained with Picrosirus Red stain were evaluated using Polarising Microscope, the colour changes were scored from 1 to 3.

1. Greenish yellow birefringence
2. Yellowish orange birefringence
3. Reddish orange birefringence.

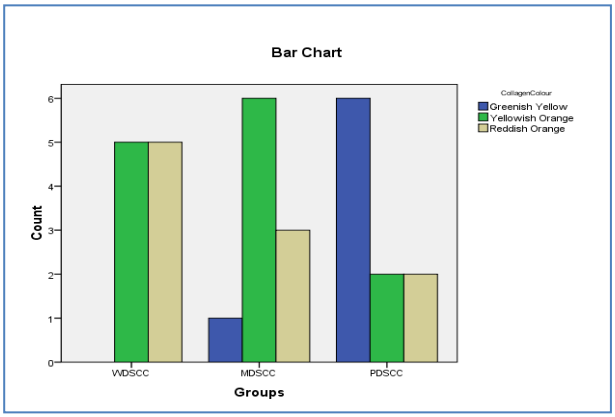
RESULTS

The Immunohistochemical study of Ki-67 showed that, Most (80%) of the well differentiated squamous cell carcinoma showed low proliferative activity, while Moderately differentiated squamous cell carcinoma showed equal proportion of both high and moderate proliferative activity (50% and 40% respectively) and Poorly differentiated squamous cell carcinoma showed high proliferative activity (70%) of malignant epithelial cells (Graph-1). In statistical analysis for Ki-67, the proliferative activity is significant with Chi-square valve 15.286,with P valve<0.005.



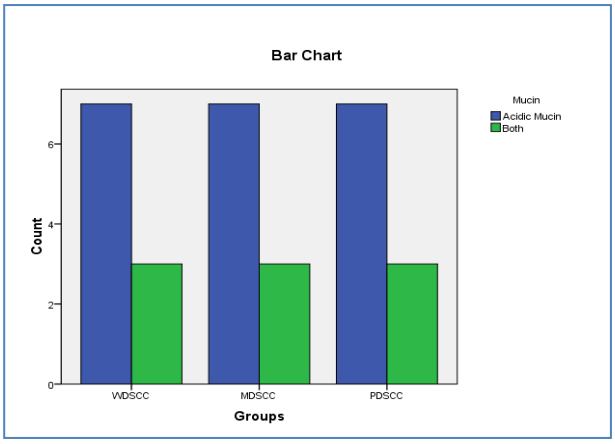
Graph 1: Bar Chart showing Ki-67 positive cells in various grades of OSCC

The nature of collagen fibres around the tumor islands was assessed using picrosirus red stain under polarising microscope. In well differentiated squamous cell carcinoma, both reddish orange (50%) and yellowish orange (50%) was seen equally. In moderately differentiated squamous cell carcinoma, more yellowish orange (60%) birefringence was noted. In poorly differentiated squamous cell carcinoma, more greenish yellow (60%) birefringence was noted (Graph-2). Chi-square test indicates that valve of 12.257 which is statistically significant with P value <0.005



Graph 2: Bar Chart showing polarasing colours of collagen fbres in various grades of OSCC

Using Alcian blue-Periodic acid Schiff reagent stain, about 7 cases (70%) showed presence of acidic mucinand 3 cases (30%) showed presence of both acidic and neutral mucin in each grade (Well differentiated, Moderately differentiated and Poorly differentiated) of squamous cell carcinoma (Graph-3). The statistical analysis with Chi-square test revealed that the presence of acidic or neutral mucin was not statistically significant in various grades of oral squamous cell carcinoma.



Graph 3: Bar Chart showing presence of acidic/neutral mucin in various grades of OSCC

DISCUSSION

Ki-67 is considered to be an important nuclear protein in cell division, as it has been observed that the antigen is expressed primarily during the cell

cycle stages of G1, S, G2 and M, with a marked emphasis on the M phase. However, Ki-67 expression is not observed during the G0 phase and has a low expression in the G1 and S phases, therefore, it is an excellent marker of cell division.[15] Scoring criteria followed in this study for Ki-67 were given by Maheswari V et al, in 2013.[16] In our study, the quantification of Ki-67 positive cells was performed in randomly selected areas, rather than invasive front of tumor or at the center of tumor sections. In the present study the expression of Ki-67 in well differentiated squamous cell carcinoma, was observed in peripheral areas of islands than the central areas of squamous maturation, which suggests that less differentiated cells are located in the peripheral layer whereas central cells are highly differentiated with the ability of keratinization, thus, no expression of Ki‑67 was observed in the central cells of the tumor island. (Fig – 1 & Fig -2) This finding correlates with the study carried out by Dwivedi N et al[17] and Birajdar SS et al.[18]

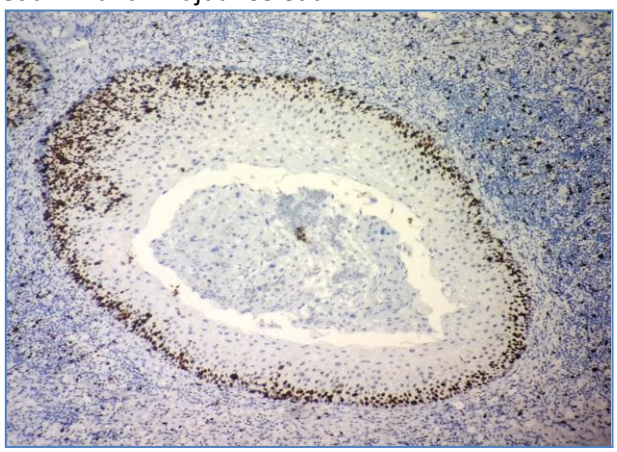


Figure 1: Ki-67 expression in peripheral areas of tumor islands in WDSCC (10x)

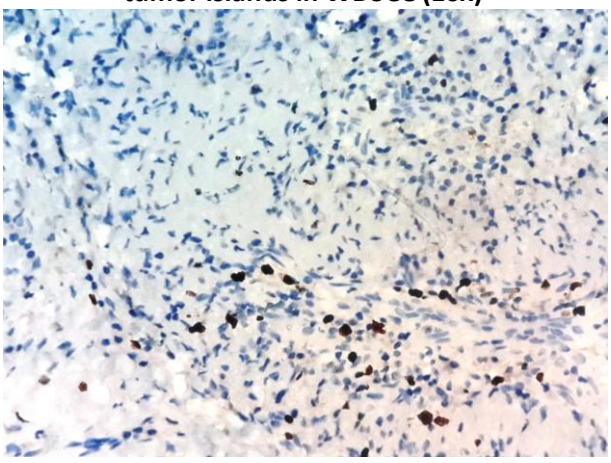


Figure 2: Expression Ki-67 in WDSCC (40x)

In moderately differentiated squamous cell carcinoma Ki‑67 expression was observed in central and peripheral areas of tumor islands and there was diffuse staining in other areas (Fig – 3). In poorly differentiated squamous cell carcinoma, the staining was more diffuse and patchy (Fig – 4), showing more proliferation rate when compared to moderately differentiated squamous cell carcinoma and well differentiated squamous cell carcinoma.

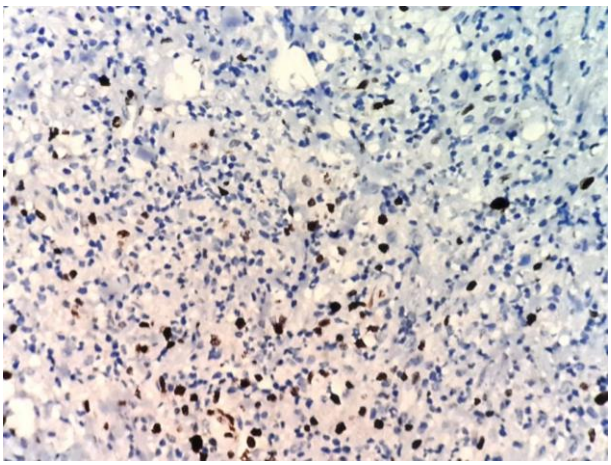


Figure 3: Expression Ki-67 in MDSCC (40x)

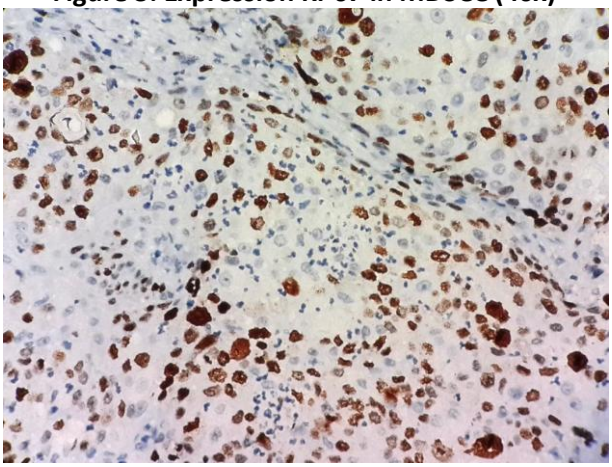


Figure 4: Expression of Ki-67 in PDSCC (40x)

These results indicates that there is significant correlation between the degree of tumor differentiation and the rate of cell proliferation as obtained by expression of Ki-67, showing that poorly differentiated squamous cell carcinoma shows high number of Ki-67 positive cells, which is found to be statistically significant in our study. The present study showed that, Ki-67 is a good proliferative marker in indicating the tumor behaviour and is also useful in predicting the prognosis of the patients in oral squamous cell carcinoma. It also shows that there is positive correlation with histological grading of oral

squamous cell carcinoma, which correlates with studies carried out by Kim et al,[19] Da Ros Mossa R et al,[20] Premalatha BR et al,[21] Maheswari V et al [16] and Patel SM et al.[22]

Collagen, a main component of the extra cellular matrix (ECM) plays a vital role in maintaining the structural integrity and tissue functioning of our body.[11] 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.[23] Picrosirius red stain is considered to be highly specific and selective stain for collagen fibers due to its ability in differentiating between different types of collagen fibers in various pathological conditions.[11] Cancer associated fibroblasts have been shown to alter the phenotype of malignant epithelial tumor cells and enhance tumor progression.[23] In the present study, the collagen fibres in well differentiated squamous cell carcinoma using picrosirus red stain, under Polarising Microscope, revealed the polarizing colours of reddish orange to yellowish orange, which was mainly concentrated around the tumor islands (Fig -5). This may be due to the deposition of collagen fibres which were in the form of thick bands and composed of closely packed fibrils, this feature being consistent with the concept of Junqueira et al [24], who stated that the thick fibres were Type I collagen fibres and exhibited an intense red birefringence. Similar results were also given in the study conducted by Aparna V et al [25] and Kalele KP et al.[26]

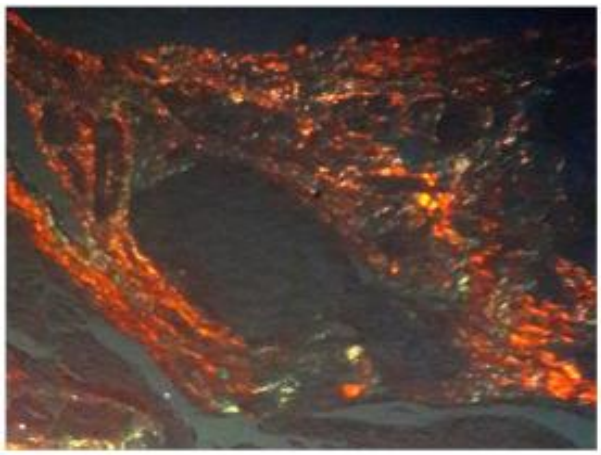


Figure 5: Photomicrograph showing collagen fibers in reddish orange colour under polarizing microscopy in WDSCC (10X)

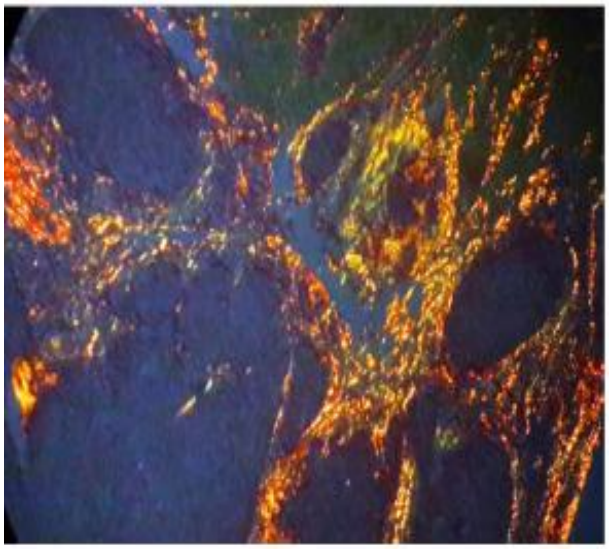


Figure 6: Photomicrograph showing collagen fibers in yellowish orange colour under polarizing microscopy in MDSCC(10X)

In moderately differentiated carcinoma, more yellowish orange birefringence (Fig -6) was observed, and in poorly differentiated squamous cell carcinoma, the predominant polarizing colour observed was greenish yellow with weak birefringence around the tumour islands.(Fig-7).The gradual change in the polarizing colours from reddish orange to greenish yellow from well to poorly differentiated SCC, indicates that as the tumor progresses, there is a change from mature collagen to an immature collagen form. This correlates with the study carried out by Aparna V et al [25] and Manjunath BS et al.[11]

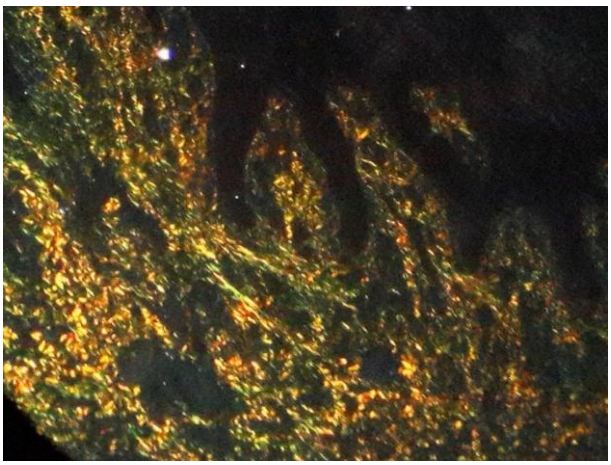


Figure 7: Photomicrograph showing collagn fibers in greenish yellow colour under polarizing microscopy in PDSCC (10X)

Junqueira et al [24] (1979) also stated that type III collagen appeared as thin weakly birefringent green fiber which supports our study as collagen fibres are more disorganised in poorly differentiated squamous cell carcinoma. 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, due to tight packing of collagen, there was the difference in polarizing colours.[11] The present study indicates that there is some is statistically significant transformation of collagen fibres in the stromal tissue around the tumor cells in various grades of oral squamous cell carcinoma, which was in accordance with the study conducted by Monsky WL et al,[27] Agrawal U et al[10] showing that these can be useful in predicting the tumor behaviour.

Mucins might serve as cell-surface receptors and sensors, and conduct signals in response to external stimuli that lead to coordinated cellular responses that include proliferation, differentiation, apoptosis and secretion of specialized cellular products. [12] Mucins are hypothesized to contribute to tumour invasion by simultaneously disrupting existing interactions between opposing cells (anti-adhesion) and establishing new ligands for interaction between the invading cell and the adjoining cells (adhesion).[12] Acidic mucins containing sulpahate group are thick, viscous and help in forming of protective coat for lubrication. Neutral mucins are slightly alkaline in nature and they mainly help in reducing the pH and toxicity of substances.[13]

In the present study, all grades of oral squamous cell carcinoma showed the presence of more acidic mucins (Fig - 8) compared to presence of neutral mucins. (Fig -9) This is in contraindication with the study by George J et al,[23] which states that increased amount of neutral mucin were seen compared to acidic mucin in all grades of oral squamous cell carcinoma.

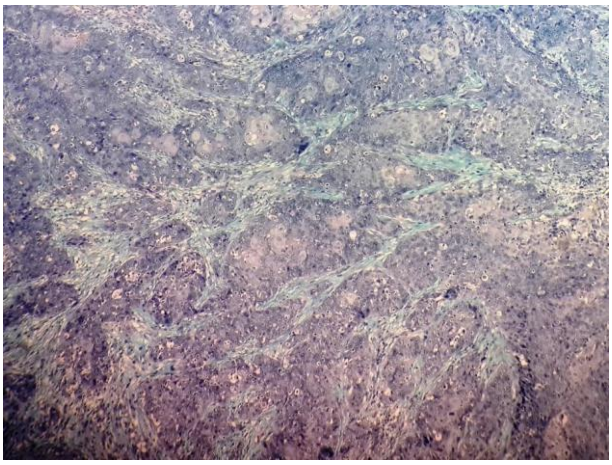


Figure 8: Photomicrograph showing acidic and neutral mucin (10x)

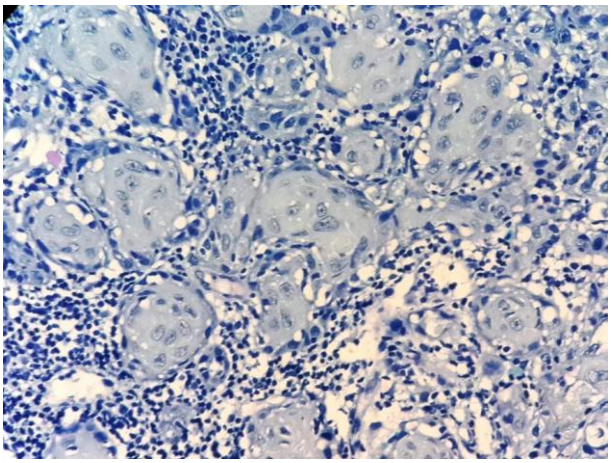


Figure 9: Photomicrograph showing acidic mucin (10x)

Mucin content and type of mucin can be used as an important prognostic indicator in colorectal cancer, as studied by Nikumbh RD et al,[13] where as in oral squamous cell carcinoma, whether the type of mucin or the mucin content can be useful in predicting the prognosis remains doubtful, as our study showed that there is no statistical significance of mucin in oral squamous cell carcinoma. However, further studies need to be performed for determining the role of mucin in oral squamous cell carcinoma.

CONCLUSION

Our present study, assessed the proliferative activity of malignant epithelial cells using Ki-67 and extracellular matrix which includes collagen (fibrous component) using Picrosirus red stain and mucin (ground substance) using combined Alcian Blue – periodic acid Schiff reagent stain in the

stroma of various grades of squamous cell carcinoma.

Tumor cell proliferation as measured by Ki-67 and Collagen birefringence changes using Picrosirus stain indicated that, there is change from mature collagen to immature form as the tumor progresses, are useful in predicting the prognosis of various grades of oral squamous cell carcinoma. The prognosis in the different grades of oral squamous cell carcinoma using Ki-67 has already proven by various authors. Comparison of Ki-67 with special stains indicates that collagen fibres can also be useful in predicting the prognosis of various grades oral squamous cell carcinoma. Presence of acidic mucin or neutral mucin, in determining the prognosis in various grades of oral squamous cell carcinoma larger samples and further studies needed.

However this study recommends that special stains can be easily done, so it can be used in assessing the prognosis of different grades of oral squamous cell carcinoma, which is cost effective and less time consuming compared to Immunohistochemistry.
In addition to routine Haematoxylin & Eosin stain, we can use special stains to determine the prognosis of different grades of oral squamous cell carcinoma, which will be helpful for treatment purpose.

BIBLIOGRAPHY

1. Bhargava A, Saigal S, Chalishazar M. Histopathological Grading systems in Oral squamous cell carcinoma: A Review. J. Int Oral Health 2101;2(4):1-10.
2. Massoano J, Regateiro FS, Januario G, Ferreria A. Oral squamous cell carcinoma: Review of prognostic and predictive factor. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:67-76.
3. Markopoulos AK. Current Aspects on Oral Squamous Cell Carcinoma. Open Dent J 2012:6;126-30.
4. de Moraes M, Monteiro Maia CAD, de Almeida Freitas R, Galvão HC .Cell proliferation markers in oral squamous cell carcinoma. J Mol Biomark Diagn 2012; S2:006
5. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol let 2014;8;7-11.
6. Singh J. Histopathology of Oral Squamous Cell Carcinoma – A Review. TMU J.Dent 2014;1(4):141-4.
7. Sharma M, Sah P, Sharma SS, Radhakrishnan R. Molecular changes in invasive front of oral cancer. J Oral Maxillofac Pathol 2013;17:240-7.
8. Ziober AF, Falls EM, Ziober BL. The extracellular matrix in oral squamous cell carcinoma: friend or foe?. Head Neck 2006;28:740-9.
9. Ziober BL, Turner MA, Palefsky JM, Banda MJ, Kramer RH. Type I collagen degradation by invasive oral squamous cell carcinoma. Oral Oncol 2000;36:365-72.
10. Agrawal U, Rai H, Jain AK. Morphological and ultrastructural characteristics of extracellular matrix changes in oral squamous cell carcinoma. Indian J Dent Res 2011;22:16-21
11. 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.
12. Hollingsworth MA, Swanson BJ. Mucins in Cancer: Protection and Control of cell surface. Nat. Rev. Cancer 2004;4:45-60.
13. Nikumbh RD, Nikumbh DB, Umarji BN. Mucin histochemical study of the colon in normal and malignant lesions. Int J Health Sci Res. 2012;2(7):20-32.
14. Kufe DW. Mucins in Cancer: function, prognosis and therapy. Nat. Rev. Cancer 2009;9(12):874-85.
15. Bonhin RG, de Carvalho GM, Guimarães AC, Chone CT, Crespo AN, Altemani AM, et al. Histologic correlationof expression of Ki-67 in squamous cell carcinoma of the glottis according to the degree of cell differentiation. Braz J Otorhinolaryngol. 2014;80:290-5.
16. Maheswari V, Sharma SC, Narula V, Jain A, Alam K. Prognostic and predictive Impact of Ki-67 in Premalignant and Malignant Squamous Cell Lesions of oral cavity. Int J. Hesd Neck Surg 2013;4(2):61-5
17. Dwivedi N, Chandra S, Kashyap B, Raj V, Agarwal A. Suprabasal expression of Ki-67 as a marker for the severity of oral epithelial dysplasia and oral squamous cell carcinoma. Contemp Clin Dent 2013;4:7-12.
18. Birajdar SS, Radhika MB, Paremala K, Sudhakara M, Soumya M, Gadivan M. Expression of Ki-67 in normal oral epithelium, leukoplakic oral epithelium and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2014;18:169-76.
19. Kim SJ, Shin HJ, Jung K-Y, Baek S-K, Shin BK, Choi J et al. Prognostic Value of Carbonic Anhydrase IX and Ki-67 Expression in Squamous Cell Carcinoma of the Tongue. Jpn J Clin Oncol 2007;37(11): 812-9.
20. Da Ros motta R, Zettler CG, Cambruzzi E. Pereira jotz G, Brutti Berni R. Ki-67 and p53 correlation prognostic value in squamous cell carcinomas of

the oral cavity and tongue. Braz J Otorhinolaryngol. 2009;75(4):544-49.

21. Premalatha BR, Uma K. Analysis of Ki-67 antigen in human Oral Squamous Cell Carcinoma – An immunohistochemical study. J,Int Oral Health 2010; 2(1):9-16.

22. Patel SM, Patel KA, Patel PR, Gamit B, Hathila RN, Gupta S. Expression of p53 and Ki-67 in oral dysplasia and squamous cell carcinoma: An immunohistochemical study. Int J Med Sci Public Health 2014;3:1201-1204.

23. 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

24. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections, Histochemical J 1979;11:447-55

25. Aparna V, Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain- a histochemical study. J Clin Diagn Res 2010;4:3444-9.

26. Kalele KP, Managoli NA, Roopa NM, Kulkarni M, Bagul N, Kheur S. Assessment of collagen fiber nature, spatial distribution, hue and its correlation with invasion and metastasis in oral squamous cell carcinoma and surgical margins using Picro Sirius red and polarized microscope. J Dent Res Rev 2014;1:14-7.

27. 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.