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Original article

Laminin - A Prognostic Marker in Oral Squamous Cell Carcinoma

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Abstract

Introduction: Squamous cell carcinoma is one of the most common malignant tumours of the oral cavity and is the tenth most common cause of death worldwide. Because of the high malignant potentially these cases are detected at an advanced stage. Also the recurrence rate is high which invites challenge to the treating surgeon. Laminin, a basement membrane molecule is a reliable marker to predict dif-ferentiation and aggressiveness of the oral squamous cell carcinomas. ***Methods:*** The study was conducted in the department of pathology, S. C B Medical College, Cuttack with an aim to predict the tumour aggressiveness and thereby prognosis. A total number of 76 cases were studied using the laminin immunostaining and the results tabulated and interpreted. ***Results:*** Majority were well differentiated squamous cell carcinomas, which showed mild to moderate intra cytoplasmic staining of laminin and an intense staining at the tumour host interface, in contrast to an intense intra cytoplasmic staining in poorly differentiated squamous cell carcinoma cases. Those with lymph node metastasis and involved surgical margins also showed intense intra cytoplasmic laminin staining pointing to a higher grade tumour. ***Conclusion:*** Laminin immunostaining is definitely superior to the conventional staining procedures for the as-sessment of tumour aggressiveness and differentiation, intra-operative assessment of tumour excision margins and thus access prognosis. Recent researches highlight on Laminin antibodies, which can be used as specific chemotherapeutic agents, to check the process of tumour growth and invasion.

Keywords: ***Immunostaining, Laminin, Oral cavity***

Introduction

Oral squamous cell carcinoma is one of the most common cancers worldwide. It accounts for more than 90% of carcinomas of the oral cavity and oropharynx [1]. Being a highly invasive malignant neoplasm, is often associated with a high risk of local and distant metastatic spread and comorbidity. Also the recurrence rate is high. Because of the high malignant potentially, the tumour is usually detected in an advanced stage. So early detection, targeted therapy and checking the process of spread can improve the prognosis and patient survival. Basement membrane zone proteins produced by squamous cell carcinomas, are required for tumour growth and metastasis. Tumour associated enzymes of the basement membrane zone, required for invasion and growth, significantly modify tumour derived basement membrane zone [2]. Laminin 332 (previously called laminin 5), is a heterotrimer composed of three different laminin chains (alpha 3, beta 3 and gamma 2) and is one of the important and major components of the basement membrane [3]. Tumour cells after binding to the laminin receptors on basal membrane, are subsequently stimulated to produce metalloproteinase - 2 which causes fragmentation and degradation of the membrane, thus facilitating tumour spread [4]. More aggressive carcinomas produce more enzymes that leads to more membrane break down and invasion.

Laminin 5 Immunohistochemical staining can be used as an important prognostic marker to check the integrity of the basement membrane, assess tumour grade and invasion and also to interpret status of intra-operative surgical margins. A new horizon in the therapeutic aspect is to check tumourigenesis and metastasis by application of newer therapeutic agents like laminin specific antibodies.

The present study is aimed at to assess the expression of laminin in oral squamous cell carcinomas and thus evaluate the histological differentiation and aggressiveness.

Methods

This is a prospective study, conducted in the department of Pathology, SCB Medical College, Cuttack, Odisha during the period from July 2018 to August 2020. The present study was approved by the Institutional Ethical Committee Report (No.156), SCB Medical College, Cuttack. Written informed consent was obtained in each case. Serial number of the resected specimen, name, age, sex, OPD/IPD number, clinical presentation, clinical

diagnosis, location of the tumour, size of the tumour, involvement of the cervical or other group of lymph nodes and surgical margins, histopathological diagnosis, grading, TNM staging were noted down in the master chart spread over the excel sheet.

After entering the data in the master chart, tissue processing, paraffin wax embedding, blocking out, section cutting, fixation of the tissue on slides, routine Hematoxylin and Eosin staining and DPX mounting was done. Tumour typing and grading were done under light microscope.

Paraffin blocks, representative of adequate tumour tissue and histologically confirmed cases of squamous cell carcinoma by H & E staining, were chosen for performing Immunohistochemistry. Laminin 332 was selected as the Immunohistochemical marker.

The inclusion criteria were histopathologically confirmed cases of oral squamous cell carcinoma, presence of adequate tissue with epithelium and sufficient connective tissue for IHC staining. Cases excluded from the study were insufficient tissue, tissues with inconclusive diagnosis, cases undergone surgery, chemotherapy or radiotherapy in the past.

Tissue sections, 3 micron thick were mounted on poly-lysine coated slides and incubated at 37.C overnight. The slides were deparaffinized in xylene and rehydrated through graded proportions of alcohol, brought upto water level and subjected to antigen retrieval in microwave woven, two cycles at 96. C for 6 minutes. The tissues were then cooled to room temperature and incubated with peroxide block for 12 minutes for blocking endogenous peroxidase activity. Then treated with protein block for 10 minutes to eliminate background staining. Subsequently, the sections were incubated with primary antibody for 2 hour, followed by secondary antibody for 30 minutes. The slides were then incubated with Novolink polymer for 30 minutes and finally with freshly prepared 3, 3' - diaminobenzidine (DAB) chromogen (1 in 20 ratio) for 1-2 minutes. Finally, the slides were washed in water to remove excess DAB and counter stained with Mayer's Hematoxylin, dehydrated, cleared and mounted with DPX.

Interpretation of staining

Ten consecutive representative fields were examined in both 10x and 40x in each cases included in the study and compared with the internal control and scored. Presence of brown coloured end product was indicative of positive immunoreactivity. Normal salivary gland taken as positive control, showed expression of laminin around the basement membrane. Basement membrane of the epithelium, blood vessels, nerves and muscles were taken as internal positive control. In the tumour part, distribution of the stain was searched for around the basement membrane of malignant epithelial cell nests along with its continuity and also within the cytoplasm of the malignant cells. Using the method done by Preeti A et al (2020), a semi quantitative assessment of expression of laminin was done in all study samples. Both the area of staining and staining intensity was evaluated for obtaining accurate results.

The area of cytoplasmic staining of laminin in the tumour cells was graded based on the percentage of positive expression in each slide as: +1 = 0-25%; +2 = 25-50%; +3 = 51- 75%; +4 = 76-100%. Using the modified method proposed by Ono et al (1999) the cytoplasmic staining of laminin in the tumour cells was graded as: 3 for intensity similar to that of control, 2 for lesser intensity as the control but definitely discernible cytoplasmic staining, 1 for mild staining 0 for no stain.

The pattern of laminin staining around the islands (at the tumour-host interface) was graded as : three- for continuous linear staining with definite colour; two- for linear staining with moderate colour ; one-for weak staining, zero-for absence of any staining.

Statistical Analysis

The results of immunohistochemical staining were noted down in tabular form. The number of cases in each category were also expressed in the form of percentages. The whole data was analysed using Statistical Package for Social Sciences (SPSS) version 21 software. The categorical variables were compared to find out the association between the different variables. A value of $P < 0.05$ was taken as statistically significant .

Results

The present study included a total number of 76 histologically diagnosed cases of oral squamous cell carcinomas. Maximum number of cases were encountered between 41 to 50 years of age with a male preponderance (M: F= 6.6:1) (Figure-1). Most of the patients had the habit of tobacco chewing and alcohol 34(44.7%) followed by tobacco chewing alone 24 (31.6%) cases. Only one case (1.3%) had no addiction history. Buccal mucosa was the most common site of affection 38 (50%), followed by tongue 27(35.5%) cases and the least affected site was floor of the mouth 5 (6.6%) (Figure-2). Most of the cases 48 (63.1%) presented with a proliferative lesion, followed by an ulcerated lesion 20 (26.3%) and only 2 cases (2.6%) presented as leukoplakia. Histologically, majority 46 (60.5%) cases were well differentiated, followed by 24(31.6m%) cases of moderately differentiated and only 6 (7.9%) cases were poorly differentiated squamous cell carcinomas. An overall comparison of laminin expression revealed (Table-1), intense intra-cytoplasmic expression (Figure-3) in tumour islands and nests of 83.3% cases of poorly differentiated squamous cell carcinomas (Table-1). This showed a statistically significant p value ($p<0.05$). In contrast, 89.1% of well differentiated squamous cell carcinomas showed nil to mild expression and 87.5% cases of moderately differentiated squamous cell carcinomas showed moderate laminin expression (Figure-4).

Results of patterns of laminin expression at tumour stroma interface showed a continuous linear staining, definitely coloured in 8 (17.4%) cases in well differentiated (Figure-5) and no such patterns in either moderately or poorly differentiated squamous cell carcinomas (100%). This finding was statistically significant ($p<0.019$).

Out of the total number of 76 cases studied, 44 cases were incisional biopsies. Rest 32 were radical surgery cases of oral squamous cell carcinomas with lymph node metastasis and/ or surgical margin involvement. All these were subjected to laminin immunohistochemical staining. It was observed that 83.3% of cases with Lymph node involved by tumour cells showed moderate to intense cytoplasmic expression of laminin within tumour cells, as compared to only 50% of un-involved cases and the difference was statistically significant ($p< 0.02$)(Table-2). Continuous linear staining at tumour- stroma interface was seen in 15% of un involved cases, while none of lymph node involved cases showed intense linear staining, which was not statistically significant ($p<0.3$). Also 35.3% of oral squamous cell carcinoma cases, in which margins were involved showed intense cytoplasmic expression of laminin within the tumour cells while only 6.7% of cases showed laminin expression when margins were free of tumour that was statistically significant (p value < 0.013)(Table-3).

Laminin expression at the tumour- stroma interface was weak or absent in 76.4% cases where margins were involved while 73.3% cases showed linear staining when margins were free which was statistically significant (p value < 0.001).

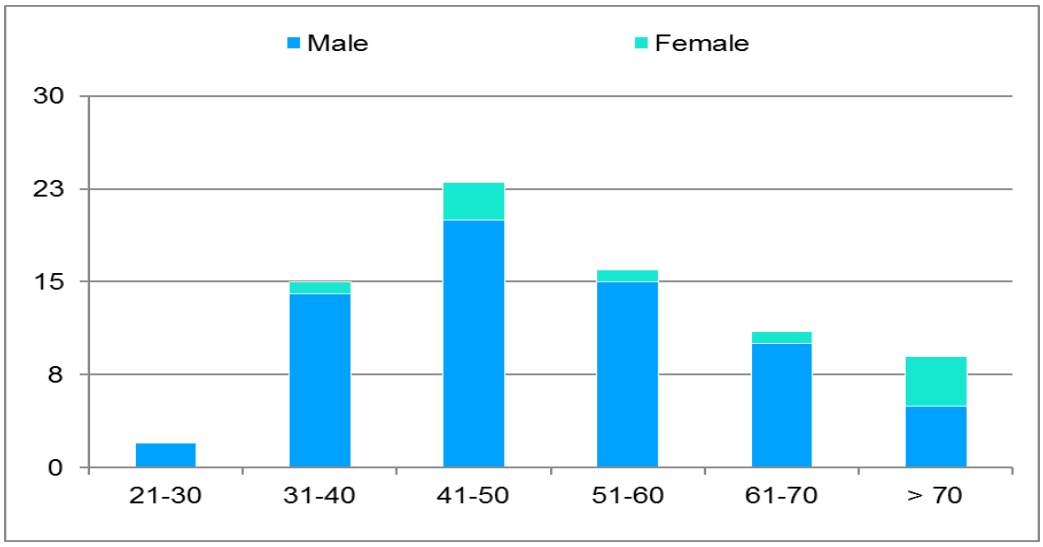

Figure 1: Age and Sex distribution of Oral Squamous Cell Carcinoma cases

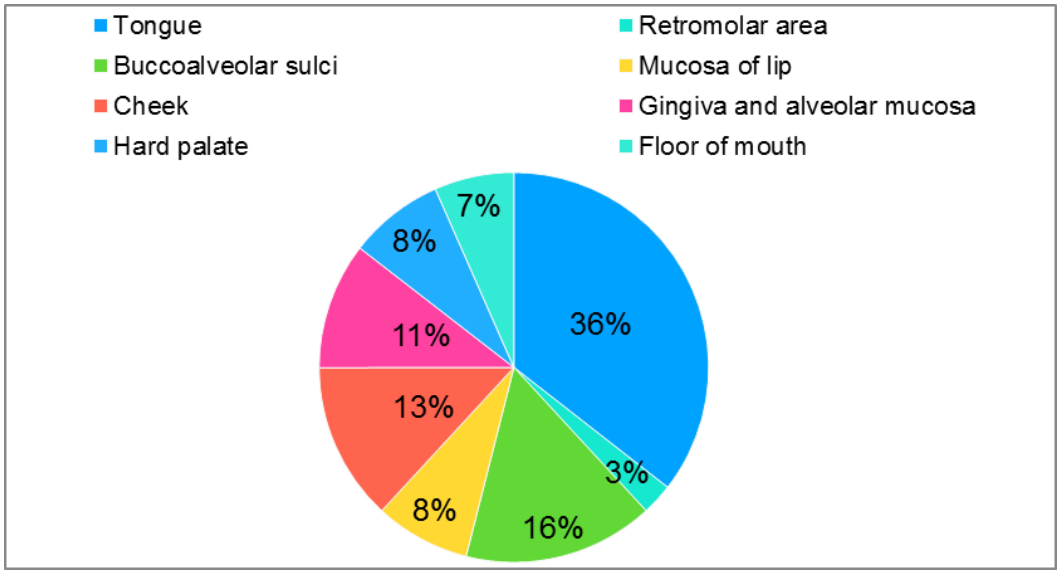

Figure 2: Sites of Oral Cavity lesions

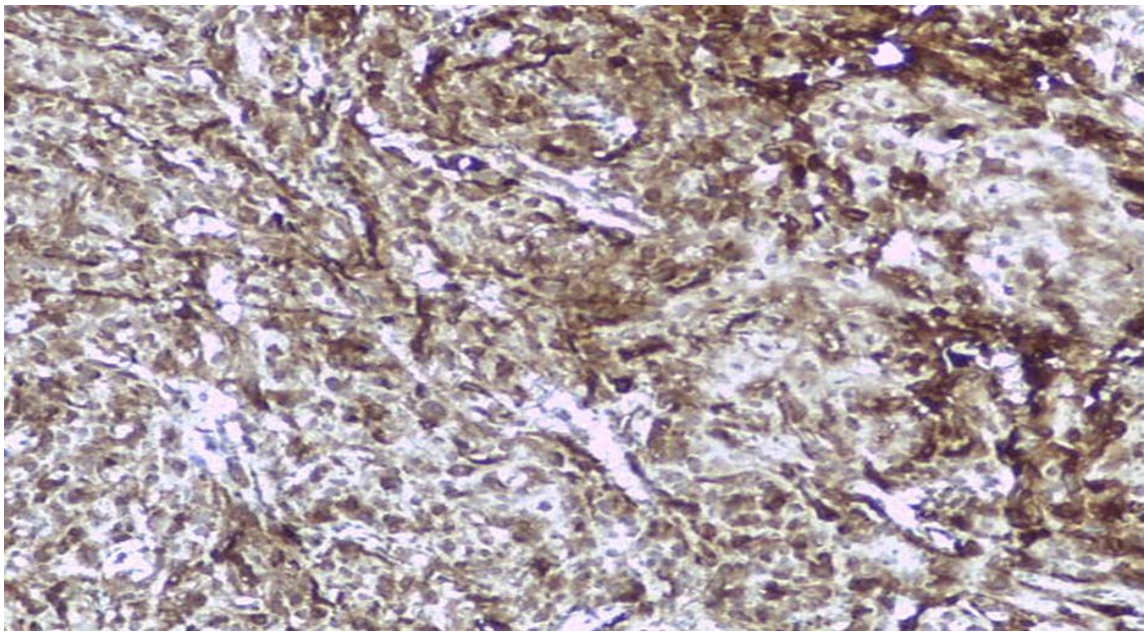

Figure 3: Microphotograph showing intense intra-cytoplasmic staining of laminin in a poor-ly differentiated squamous cell carcinoma. (x 40)

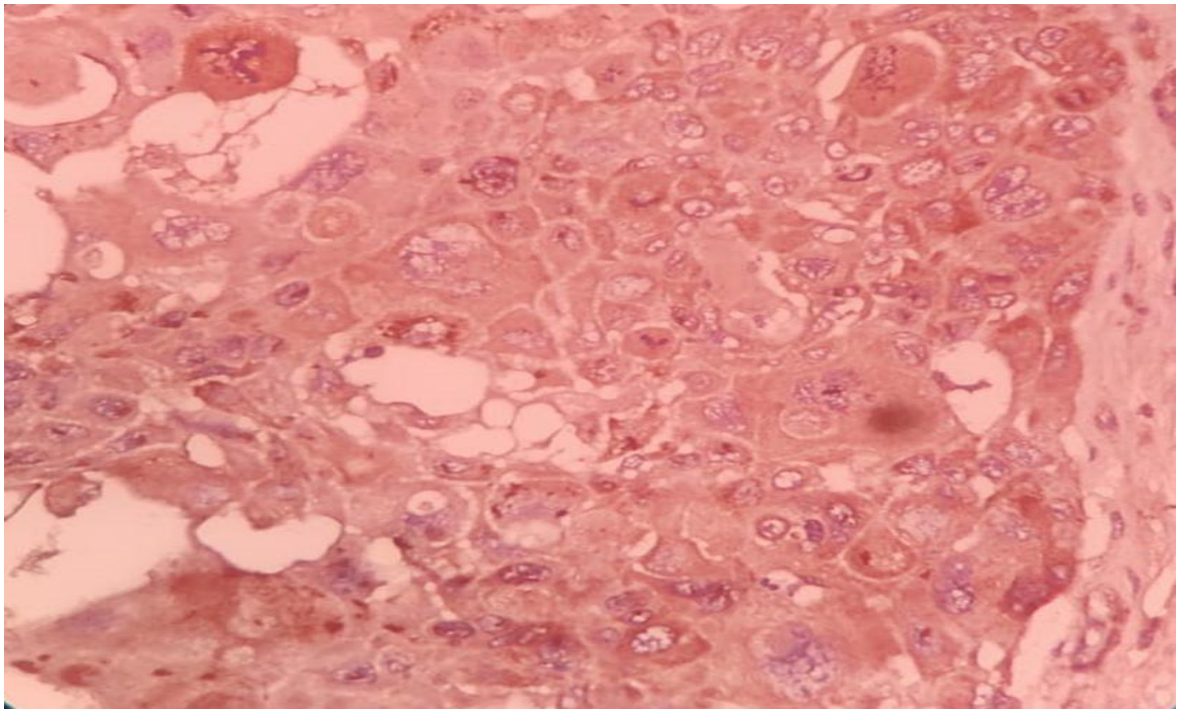

Figure 4: Microphotograph showing moderate intra-cytoplasmic staining of laminin in a moderately differentiated squamous cell carcinoma. (x 40)

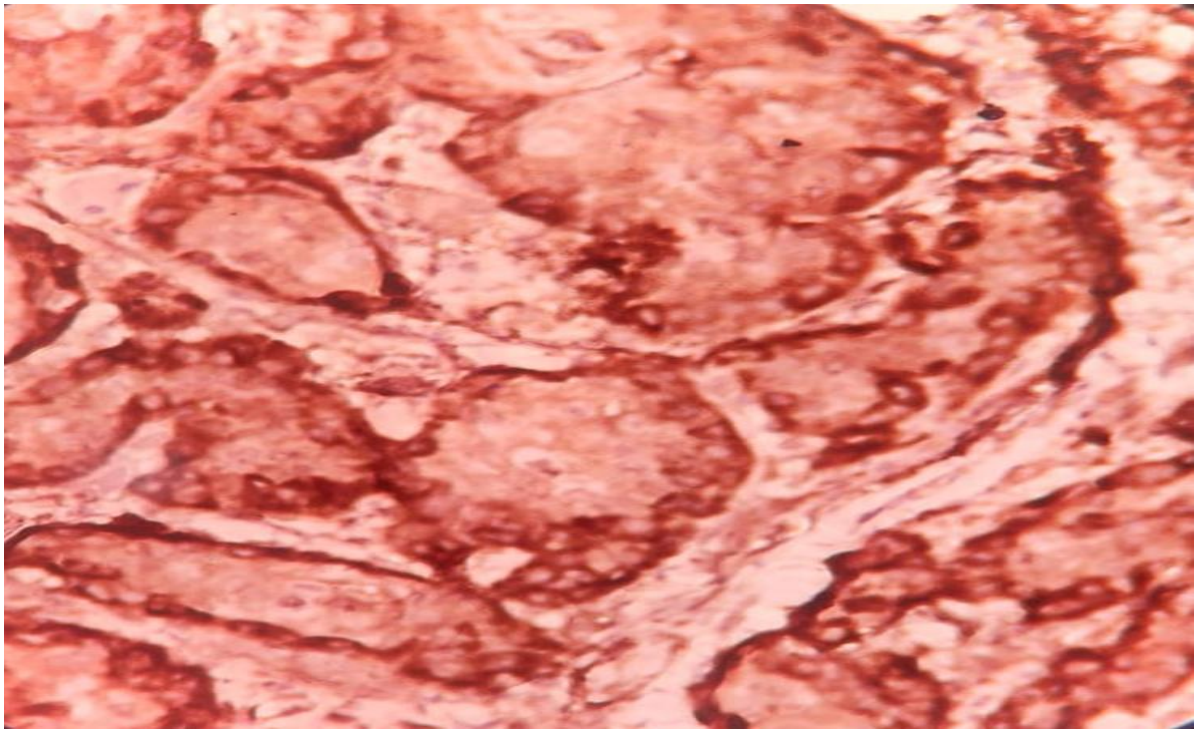

Figure 5: Microphotograph showing continuous linear staining of laminin at tumour-host interface in a well differentiated squamous cell carcinoma. (x 40)

Table 1: Expression of laminin in different histological grades of squamous cell carcinoma

Laminin Expression	**Well differentiated OSCC (n)**	**Percentage (%)**	**Moderately differentiated OSCC (n)**	**Percentage (%)**	**Poorly differentiated OSCC (n)**	**Percentage (%)**	**P value**
Cytoplasmic Expression							<0.05
a. Mild or absent	41	89.1	3	14.3	0	0	
b. Moderate	5	10.9	21	87.5	1	16.7	
c. Intense	0	0	0	0	5	83.3	
Pattern of Laminin expression at tumour stroma interface							0.019

a. No or very weak staining	27	58.7	24	100	6	100	
b. Weak staining	3	6.5	0	0	0	0	
c. Linear staining, moderately coloured	8	17.4	0	0	0	0	
d. Continuous linear staining, definitely coloured	8	17.4	0	0	0	0	

Table 2: Comparision of laminin expression in squamous cell carcinoma with involved lymph nodes

Laminin Expression	Free lymphnode (n=20)	Percentage (%)	Lymphnode involved (n=12)	Percentage (%)	P value
Cytoplasmic Expression	0.02				
a. Mild or absent	10	50	2	16.7	
b. Moderate	5	25	9	75	
c. Intense	5	25	1	8.3	
Pattern of Laminin expression at tumour stroma interface	0.3				
a. No or very weak staining	16	80	8	66.7	
b. Weak staining	1	5	2	8.3	
c. Linear staining, moderately coloured	0	0	2	16.7	
d. Continuous linear staining, definitely coloured	3	15	0	8.3	

Table 3: Comparision of laminin expression in squamous cell carcinomas with involved surgical margins

Laminin Expression	Surgical margin free (n=15)	Percentage (%)	Surgical margin involved (n=17)	Percentage (%)	P value
Cytoplasmic Expression	0.013				
d. Mild or absent	10	66.7	3	17.6	
e. Moderate	4	26.6	8	47.1	
f. Intense	1	6.7	6	35.3	
Pattern of Laminin expression at tumour stroma interface	0.001				
e. No or very weak staining	2	13.3	13	76.4	
f. Weak staining	1	6.7	2	11.8	
g. Linear staining, moderately coloured	1	6.7	2	11.8	
h. Continuous linear staining, definitely coloured	11	73.3	0	0	

Discussion

Laminin has a role in various important biological activities, like assembly of the basement membrane, cell attachment, migration, growth and differentiation, neurite outgrowth and angiogenesis. Interaction of cancer cells with laminin is a key event in tumour spread and metastasis [5]. It induces an increase in matrix metalloproteinase - 2 (an extra cellular matrix degrading endopeptidase) activity key to the invasion and metastasis. So, laminin can be used as a potential biomarker to evaluate tumour histologic differentiation and aggressiveness in oral squamous cell carcinomas [6].

In the present study, 83.3% of the poorly differentiated squamous cell carcinoma cases showed intense intra-cytoplasmic staining of laminin in the tumour cells (statistically significant); whereas well differentiated and moderately differentiated squamous cell carcinoma cases showed mild to moderate expression. Ono et al showed high invasiveness and poor prognosis in cases of squamous cell carcinoma of tongue associated with intense cytoplasmic expression of laminin [7]. Patel et al also observed over expression of laminin confined to cytoplasm in cases of oral squamous cell carcinoma [8]. The higher cytoplasmic expression in poorly differentiated squamous cell carcinoma may be due to overproduction of laminin gamma 2 monomer during carcinogenesis, that prevents export of other laminin chains and adds to intra cytoplasmic accumulation (Koshikawa et al 1999) [9] or due to internalisation of the laminin receptor and ligand by an actively invading carcinoma cell (Wewer et al 1987) [10]. Also continuous linear staining with definite colour was seen at the tumour stroma interface in 17.4% cases of well differentiated squamous cell carcinoma (statistically significant) and absent in both moderately and poorly differentiated squamous cell carcinomas. This result correlates with that of Souza et al and Mostafa et al [11,12]. This is probably due to retention of some ability of the tumour cells to produce basement membrane components in well differentiated malignant cells (Shruthyet al 2013) [13]. We observed that, 83.3% of cases with lymph node ifnfiltration by tumour cells showed moderate to intense cytoplasmic expression of laminin within tumour cells, as compared to 50% of un- involved cases, which was statistically significant (p value < 0.02). Continuous linear staining was seen at tumour- host interface in 15% of uninvolved cases, while none of the lymph node involved cases showed linear staining which was statistically insignificant. This may be due to the fact that few of the poorly differentiated tumours retain the ability of basement membrane production as reported be some authors. Present study revealed that 35.3% of cases showed intense cytoplasmic staining,

in which margins were involved, while only 6.7% cases showed intense cytoplasmic staining where margins were free,(statistically significant). At the tumour- host interface, the laminin expression was weak or absent in 76.4% of margin- involved cases, while 73.3% cases showed weak staining where margins were free (statistically significant). A pattern of higher cytoplasmic laminin expression was observed in cases of involved surgical margins and lymph node metastasis along with weak to absent linear staining around tumour host interface. Thus, laminin seems to be clinically associated with various clinico- pathological parameters indicative of invasion and metastasis. Higher laminin-5 gamma 2 expression is associated with high- intensity tumour budding and invasion[14].

Laminin 5 can also be used as a potential marker for the intra operative assessment of cancer excision margins, as it is expressed only in oral squamous cell carcinoma and not in dysplastic lesions. Several literatures have also suggested the potential role of laminin-5 not only in oral squamous cell carcinoma [15], but also in breast cancer [16], cervical adenocarcinoma and several others.

So researches in the field of preparing cancer chemotherapeutic agents like the laminin antibodies is at its pace. Identification of other domains in laminin 5, which selectively affect squamous cell carcinoma progression would be an important goal in designing future cancer therapies. Also, as laminin gamma 2 chain plays an important role in tumour progression, targeting of astacin- like laminin processing enzymes may be used in developing future anticancer therapies.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethical Committee Report (No.156), SCB Medical College, Cuttack. Written informed consent was obtained in each case.

List of abbreviations

Tumour Node Metastasis (TNM)

Statistical Package for Social Sciences (SPSS)

Hematoxylin and Eosin (H&E)

Immunohistochemical (IHC)

Dibutylphthalate Polystyrene Xylene (DPX)

Data Availability

Data shall be provided by the corresponding author on request.

Conflicts of Interest

We declare that there is no conflict of interest regarding the publication of this paper.

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The authors did not receive any funding for this work.

Authors' contributions

SS processed the specimens from the patients and reported back the results. AP analysed and interpreted the data. GR was instrumental in reviewing the literature, compiling the data and writing the manuscript. All authors read and approved the final manuscript.

Conclusion

Researches in the field of early detection, diagnosis and therapeutic interventions have been intensified currently, keeping in mind the high incidences world wide, the aggressiveness and poorer prognosis of oral squamous cell carcinomas. Recent studies have explored the role of basement membrane molecules as a major contributor for oral squamous cell carcinoma development and progression. Alterations in extra cellular matrix proteins, in particular laminin 332gamma 2 chain influences cell adhesion, differentiation and migration or spread and metastasis. So laminin 332 can now be used as a key and important marker, for evaluation of differentiation and aggressiveness of oral carcinomas. Adding to this, studies have shown that laminin can be used as a marker for intra-operative assessment of cancer excision margins. A new horizon, in the aspect of treatment of oral squamous cell carcinomas, is the use of specific laminin antibodies, as chemotherapeutic agents, for anticancer therapy. This will no doubt help prevent further spread of malignancy, improve patient survival and prognosis.

References

[1] Rao SV, Mejia G, Roberts- Thomson K, Logan R. Epidemiology of oral cancer in Asia in the past decade- an update (2000- 2012). Asian Pacific J Cancer Prev 2013;14:5567-5577.

[2] Marinkovich MP. Tumour microenvironment: Laminin 332 in squamous cell carcinoma. Nat Rev Cancer 2007; 7(5):370-80.

[3] Nguyen BP, Ryan MC, Gil SG, Carter WG 2000: Deposition of Laminin 5 in epidermal wounds regulate integrity signaling and adhesion. Curr. Opin.Cell Biol. 12(5):554-562.

[4] Sharma M, Sah P, Sharma SS, Radhakrishnan R 2013. Molecular changes in invasive front of oral cancer. J Oral Maxillofac Pathol, 17, 240-7.

[5] Pupa SM, Menard S, Forti S, Tagliabue E, 2002. New insights into the role of extracellular matrix during tumor onset and progression. J. Cell Physiol, 192: 259-267.

[6] Preeti A, Gokul S, Divyesh W, Sangeeta P. Immunohistochemical evaluation of laminin 5 and myofibroblasts in epithelial dysplasia and oral squamous cell carcinoma.IJMBS, vol 4, issue 3, 2020, 172-177.

[7] Ono Y, Nakanishi Y, Ino Y et al. Clinico- pathologic significance of laminin 5 gamma 2 chain expression in squamous cell carcinoma of the tongue: immunohistochemical analysis of 67 lesions.1999. Cancer, 85, 2315- 21.

[8] Patel V, Aldridge K, Easley JF, et al (2002). Laminin gamma 2 over expression in head and neck squamous cell carcinoma. Int J Cancer. 99, 583- 88.

[9] Koshikawa N, Moriyama K, Takamura H, et al. Over expression of laminin gamma 2 chain monomer in invading gastric carcinoma cells.1999. Cancer Res. 59, 5596- 601.

[10] Wewer UM, Taraboletti G, Sobel ME, Alberchtsen R, Liotta LA (1987). Role of laminin receptor in tumour cell migration. Cancer Res, 47, 5691-8.

[11] Souza LF, Souza VF, Silva LD, Santos JN, Reis SR, (2007). Expression of laminin in oral squamous cell carcinoma. Braz J Otolaryngol, 73, 768- 74.

[12] Mostafa WZ, Mahfouz SM, Bosseila M, Sobhi RM, Zaki NS. An immunohistochemical study of laminin in cutaneous and mucosal squamous cell carcinoma. J Egypt Women Dermatol Soc 2007; 4: 24-33.

[13] Shruthy R, Sharada P, Swaminathan U, Nagamalini BR (2013). Immunohistochemical expression of basement membrane laminin in histological grades of oral squamous cell carcinoma. A semi quantitative analysis. J Oral Maxillofac Pathol. 17, 185- 9.

[14] Marangon JH, Rocha VN, Leite CF, de Agular MC, Souza PE, Horta MC. Laminin-5 gamma 2 chain expression is associated with intensity of tumor budding and density of stromal myofibroblasts in oral squamous cell carcinoma. J Oral Pathol Med 2014; 43(3):199-204.

[15] Yellapurkar S, Natarajan S, Boaz K, Manaktala N, Baliga M, Shetty P et al. Expression of laminin in Oral Squamous Cell Carcinomas. Asian PAC J Cancer Prev 2018;19:407-413.

[16] Quick X, Tan H, Fu D, Zhu Y, Zhang J. Laminin is over expressed in breast cancer and facilitate cancer cell metastasis. J Cancer Res Ther. 2018 Dec;14(Supplement): S1170-S1172.