

## ORIGINAL ARTICLE

# Immunohistochemical expression of basement membrane laminin in histological grades of oral squamous cell carcinoma: A semiquantitative analysis

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## ABSTRACT

**Background and Objectives:** To determine the immunohistochemical (IHC) localization of basement membrane component laminin in histological grades of oral squamous cell carcinoma (OSCC). The purpose of this study was to demonstrate the loss of continuity of the basement membrane in OSCC using an antibody directed against laminin using advanced polymer staining system. **Materials and Methods:** A total of 30 cases of OSCC: 10 cases of well differentiated squamous cell carcinoma (WDSCC), 10 cases of moderately differentiated squamous cell carcinoma (MDSCC), and 10 cases of poorly differentiated squamous cell carcinoma (PDSCC) were subjected to heat-induced antigen retrieval method using ethylene-di-amine-tetraacetic acid buffer in a microwave oven. Then the sections were stained with anti-laminin polyclonal antibody and visualized using super sensitive polymer horseradish peroxidase detection system. In each case, the integrity of the basement membrane laminin was assessed by using statistical analysis. **Results:** Statistical analysis showed a decreased distribution of laminin from WDSCC to MDSCC to PDSCC (*P* value 0.0573). The intracytoplasmic staining of laminin gradually increased from WDSCC to MDSCC to PDSCC (*P* value 0.0198). **Interpretation and Conclusion:** WDSCC cases showed more laminin expression in basement membrane around the tumor islands and less loss of continuity compared to MDSCC and PDSCC cases suggesting a greater enzymatic degradation of basement membrane components in MDSCC and PDSCC than WDSCC. The loss of structural basement membrane laminin and the presence of laminin in the tumor cells of PDSCC cases suggest that laminin helps in tumor invasion. The expression of laminin in the basement membrane may be a useful parameter to evaluate tumor histologic differentiation and aggressiveness.

**Key words:** Basement membrane, immunohistochemistry, laminin, oral squamous cell carcinoma, tumor invasion

## INTRODUCTION

Squamous cell carcinoma (SCC) is defined as “a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges”.<sup>[1]</sup>

The etiology of oral cancer is almost certainly multifactorial. The carcinogenic changes may be influenced by oncogenes, tumor suppressor genes, carcinogens, and mutations caused by chemicals, viruses, irradiation, drugs, tobacco, alcohol, hormones, nutrients, or physical irritants.<sup>[2]</sup>

Laminin is a large (900 kDa) mosaic protein which is a component of the basement membrane and is composed of many distinct domains with different structures and functions. Laminins are large heterotrimeric extracellular glycoproteins composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits.<sup>[3]</sup>

Laminin 5 ( $\alpha$ 3,  $\beta$ 3,  $\gamma$ 2) is a typical component of epithelial basement membrane and is considered as a biochemical

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10.4103/0973-029X.119755

equivalent of the anchoring filaments fixing basal keratinocytes to the basement membrane.<sup>[4]</sup>

Basement membrane degradation must occur for most tumors to invade tissues and is essential for metastasis. As a readily detectable basement membrane component, laminin can be used as a marker for presence of intact or degraded basement membrane during tumorigenesis.<sup>[5]</sup>

The purpose of this study is to demonstrate the break in the continuity of the basement membrane and correlate the same with the histological grading.

Oral SCC is one of the most invasive human tumors. Unravelling how laminin influences tumor development and invasion is fundamental in the development of new prognostic indicators and treatment strategies for OSCC.<sup>[5]</sup>

## MATERIALS AND METHODS

Thirty cases of OSCC were retrieved from the archives of our college which included 10 cases of WDSCC, 10 cases of MDSCC and 10 cases of PDSCC oral squamous cell carcinoma. Normal salivary gland tissue was used as control. All the blocks were sectioned using a semiautomatic microtome (Microm HM340E) and subjected to immunohistochemical (IHC) study using polyclonal antihuman laminin primary antibody.

### Immunohistochemical procedure followed for laminin staining

Tissue sections 3 µm thick mounted on poly-lysine coated slides were incubated at 37°C overnight and then for an hour at 60°C before staining. The slides were deparaffinized in xylene and rehydrated through graded alcohol into water and subjected to antigen retrieval using microwave oven, two cycles at 96°C for 6 min. The tissues were cooled to room temperature and incubated with peroxide block for 12 min to block endogenous peroxidase activity and subsequently for 10 min with protein block to eliminate background staining. The sections so treated were then incubated with primary antibody for 2 h followed by post primary for 30 min. Subsequently, they were incubated with Novolink polymer for 30 min and finally with fresh 3,3'-diaminobenzidine (DAB) chromogen for 1-2 min (prepared in a ratio of 1:20). The slides were then washed in water to remove the excess DAB and counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted with DPX and assessed for staining characteristics. Tris buffer was used as wash buffer as and when required.

### Interpretation of staining

Normal salivary gland tissue showed positive expression around the basement membrane. [Figure 1] Presence of brown colored end product was indicative of positive

immunoreactivity. Basement membranes of the epithelium, blood vessels, nerves, and muscles were used as internal positive control. The distribution of the stain in each case of OSCC was observed in the following areas: around the basement membrane of malignant epithelial islands and its continuity and within the cytoplasm of the cells.

## RESULTS

The present study included a total of 30 confirmed cases of OSCC of which 10 cases were WDSCC, 10 cases MDSCC, and 10 cases PDSCC.

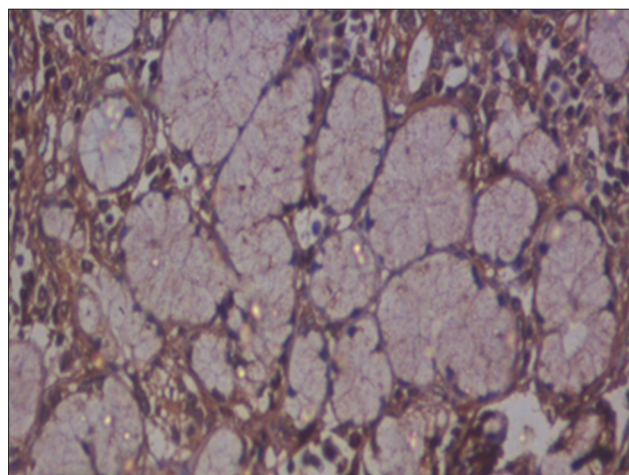
In each case, the integrity of the basement membrane laminin and intracytoplasmic laminin was assessed and subjected to appropriate statistical analysis.

The number of positive and negative cases in each parameter (basement membrane, continuity around the basement membrane and intracytoplasmic staining) under each grade (well, moderate, and poor) were tested for significance using binomial test and compared using the Fisher exact test.

Table 1 shows the results of parameters in WDSCC, where 90% cases were positive for laminin around the basement membrane out of which 80% showed continuous positivity and was statistically significant [Figure 2 and 3]. Thirty percent of the cases showed intracytoplasmic positive staining [Figure 4].

Table 2 shows the results of parameters in MDSCC, where 80% of the cases were positive for laminin both around the basement membrane and within the cytoplasm [Figure 5 and 6]. Eighty percent showed continuous positivity around the basement membrane. These results were statistically significant.

Table 3 details the results of parameters in PDSCC. In PDSCC cases, 40% were positive for laminin around the basement membrane of which 30% showed continuous positivity.



**Figure 1:** Photomicrograph of control tissue: Positive expression of laminin around the basement membrane of salivary gland (IHC stain, ×200)

Ninety percent of the cases showed intracytoplasmic positive staining which was statistically significant [Figure 7].

Table 4 compares the expression of laminin around the basement membrane between the three grades. The expression was statistically significant between WDSCC and PDSCC cases.

Table 5 compares the continuous expression of laminin around the basement membrane between the three grades which was not statistically significant.

Table 6 compares the intracytoplasmic staining between the three grades. Comparison between WDSCC and PDSCC cases showed statistical significance.

Comparison of all the three parameters in different grades of OSCC is shown in Figure 8.

## DISCUSSION

OSCC is the most prevalent malignant neoplasm. Despite the ready accessibility of the oral cavity to direct examination, these malignancies are often still not detected until it is

**Table 1: Results of parameters in well-differentiated oral squamous cell carcinoma**

Parameter	Levels	Count	P value	Result
Around the basement membrane	Positive	9	0.0057	Very significant
	Negative	1		
Continuity around the basement membrane	Positive	8	0.0289	Significant
	Negative	2		
Intracytoplasmic staining	Positive	3	0.1030	Not significant
	Negative	7		

**Table 2: Results of parameters in moderately differentiated oral squamous cell carcinoma**

Parameter	Levels	Count	P value	Results
Around the basement membrane	Positive	8	0.0289	Significant
	Negative	2		
Continuity around the basement membrane	Positive	8	0.0289	Significant
	Negative	2		
Intracytoplasmic staining	Positive	8	0.0289	Significant
	Negative	2		

**Table 3: Results of parameters in poorly differentiated oral squamous cell carcinoma**

Parameter	Levels	Count	P value	Results
Around the basement membrane	Positive	4	0.2635	Not significant
	Negative	6		
Continuity around the basement membrane	Positive	3	0.1030	Not significant
	Negative	7		
Intracytoplasmic staining	Positive	9	0.0057	Very significant
	Negative	1		

very late and as a result, the survival rate for oral cancer has remained essentially unchanged over the past decades. In recent years, numerous prognostic factors associated with OSCC have been identified.<sup>[6]</sup>

Basement membrane is a dynamic structure that undergoes quantitative and qualitative changes during the progression of SCC, which is essentially important in tumoral invasion and metastasis.<sup>[7]</sup>

Laminin is a glycoprotein that is present in the basal membrane and has specific actions including an adhesion function. Tumor cells bind to laminin receptors on the basement membrane and are subsequently stimulated to produce metalloproteinases, which begin fragmentation and degradation of the membrane and help in tumor invasion.<sup>[7]</sup>

The aim of the present study was to demonstrate the expression of laminin in different grades of OSCC. All the 30 cases of OSCCs, 10 in each category were stained using the polyclonal laminin antibody and assessed for positive and continuous expression around the basement membrane and within the cytoplasm.

Positive laminin expression around the basement membrane was seen in 90% cases of WDSCC, 80% cases of MDSCC,

**Table 4: Comparison of parameters (around the basement membrane) between the three grades**

Around the basement membrane	P value	Result
WDSCC vs. MDSCC	1.0000	Not significant
WDSCC vs. PDSCC	0.0573	Moderately significant
MDSCC vs. PDSCC	0.1698	Not significant

WDSCC: Well-differentiated oral squamous cell carcinoma, MDSCC: Moderately differentiated oral squamous cell carcinoma, PDSCC: Poorly differentiated oral squamous cell carcinoma

**Table 5: Comparison of parameters (continuity around the basement membrane) between the three grades**

Continuity	P value	Result
WDSCC vs. MDSCC	1.0000	No significance
WDSCC vs. PDSCC	0.0698	No significance
MDSCC vs. PDSCC	0.0698	No significance

WDSCC: Well-differentiated oral squamous cell carcinoma, MDSCC: Moderately differentiated oral squamous cell carcinoma, PDSCC: Poorly differentiated oral squamous cell carcinoma

**Table 6: Comparison of parameters (intracytoplasmic staining) between the three grades**

Intracytoplasmic staining	P value	Result
WDSCC vs. MDSCC	0.0698	No significance
WDSCC vs. PDSCC	0.0198	Moderate significance
MDSCC vs. PDSCC	1.0000	No significance

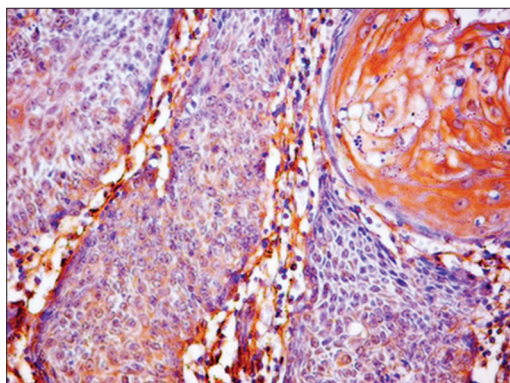
WDSCC: Well-differentiated oral squamous cell carcinoma, MDSCC: Moderately differentiated oral squamous cell carcinoma, PDSCC: Poorly differentiated oral squamous cell carcinoma

and 40% cases of PDSCC. The expression was continuous around the basement membrane in 80% of WDSCCs and MDSCCs, which was statistically significant. Only 30% of PDSCCs showed continuous positive expression around the basement membrane which was not statistically significant.

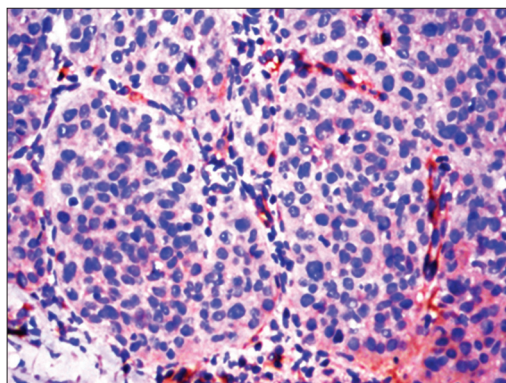
These results are in accordance with previous studies by Tosios *et al.*,<sup>[8]</sup> Souza *et al.*,<sup>[7]</sup> and Mostafa *et al.*,<sup>[9]</sup> where positive continuous staining of laminin was observed around the basement membrane. This indicates that the cells adjacent to basement membrane and the tumor cells secrete laminin.

Intracytoplasmic staining was observed in all the grades of OSCC. 30% of WDSCCs, 80% of MDSCCs, and 90% of PDSCCs showed positive staining indicating a statistical significance in case of MDSCC and PDSCC. This may be related to increased production and reduced secretion of laminin by the cells as they become progressively anaplastic.<sup>[10]</sup>

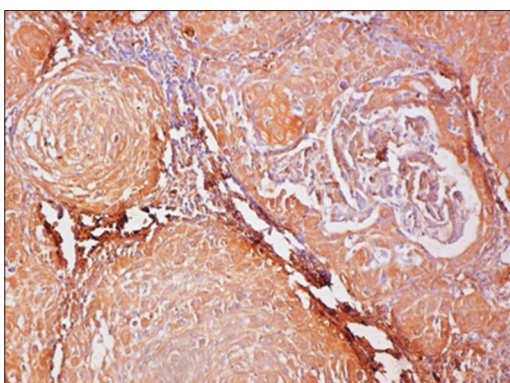
This is in accordance with the study by Mostafa *et al.*,<sup>[9]</sup> where varied intracytoplasmic staining was observed in all the grades.



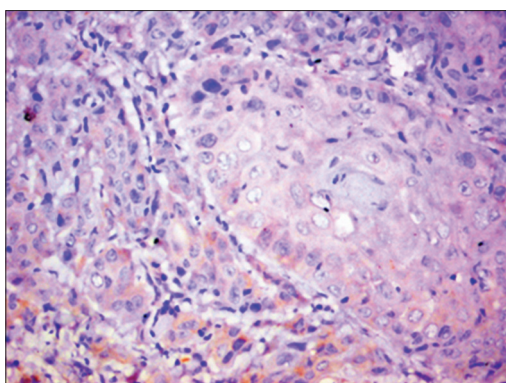
**Figure 2:** Positive expression of laminin around the basement membrane in WDSCC (IHC stain, ×200)



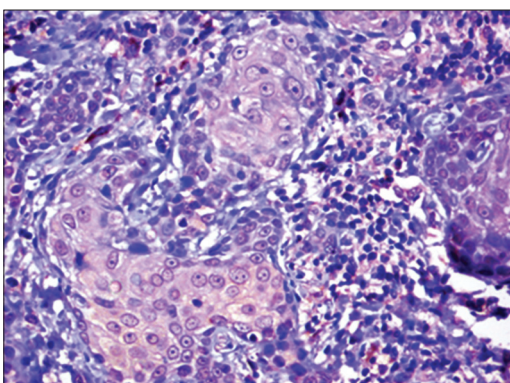
**Figure 3:** Loss of continuity of expression of laminin around the basement membrane in WDSCC (IHC stain, ×200)



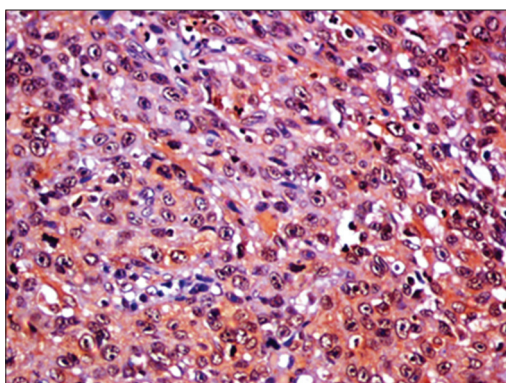
**Figure 4:** Positive intracytoplasmic staining for laminin in WDSCC (IHC stain, ×200)



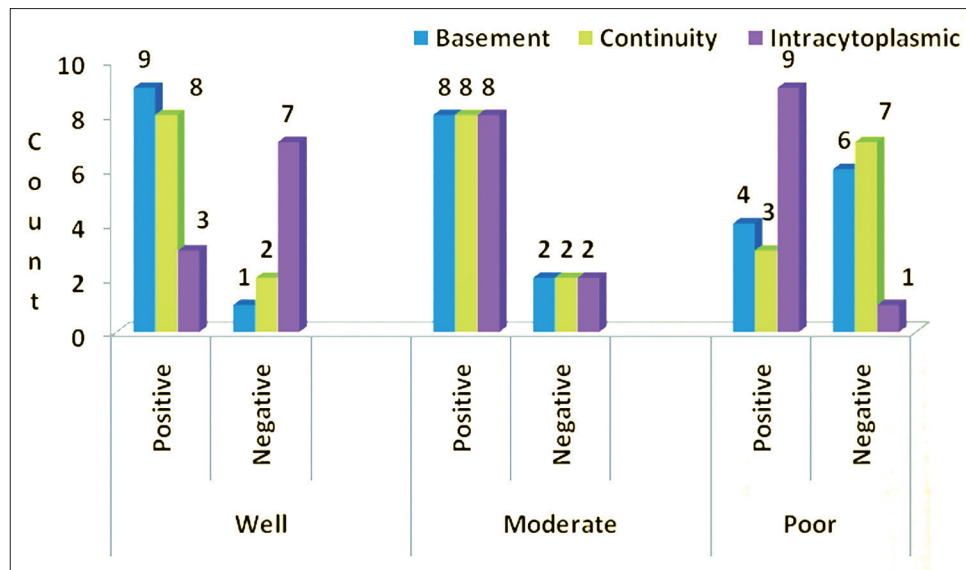
**Figure 5:** Loss of continuity of expression of laminin around the basement membrane in MDSCC (IHC stain, ×200)



**Figure 6:** Positive intracytoplasmic staining for laminin in MDSCC (IHC stain, ×200)



**Figure 7:** Positive intracytoplasmic staining for laminin in PDSCC (IHC stain, ×200)



**Figure 8:** Graph showing comparison of all the three parameters in different grades of oral squamous cell carcinoma

Similar studies have been carried out previously. Firth and Reade<sup>[11]</sup> showed that laminin distribution was continuous in epithelial hyperplasia while dysplastic lesions showed small focal breaks whose number increased in severe dysplasia. Kannan *et al.*,<sup>[12]</sup> reported a gradual increase in the frequency of laminin discontinuity from normal epithelium to hyperplastic, dysplastic and SCCs, with significant differences among groups. Harada *et al.*, found that the staining pattern of laminin in primary OSCCs was similar to that of metastatic nodules. Santos-García *et al.*,<sup>[13]</sup> found discontinuity in dysplastic lesions that progressively increased for *in situ* and microinvasive carcinomas, being higher in OSCCs and total in the group of metastatic nodules. Our findings confirm and support these studies.

## CONCLUSION

We conclude from our study that, WDSCC cases showed more laminin expression in tumor island basement membrane and less loss of continuity compared to PDSCC cases suggesting a greater enzymatic degradation of basement membrane components than PDSCC.

Therefore, the expression of laminin in the basement membrane may be a useful parameter to evaluate tumor histologic differentiation and aggressiveness. The absence of staining is associated with poor prognosis.

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**How to cite this article:** Shruthy R, Sharada P, Swaminathan U, Nagamalini BR. Immunohistochemical expression of basement membrane laminin in histological grades of oral squamous cell carcinoma: A semiquantitative analysis. J Oral Maxillofac Pathol 2013;17:185-9.

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