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Basement Membrane Alterations in Oral Lichen Planus and Oral Squamous Cell Carcinoma: Correlation with Laminin 5 Gamma 2 Expression

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Abstract
Oral lichen planus (OLP) is a prevalent chronic inflammatory disease that primarily affects middle-aged women. There is a substantial chance that OLP lesions will acquire a cancerization field and undergo malignant transformation. Laminin 5 gamma 2, a key component of the epithelial basement membrane, was studied in oral lichen planus and oral squamous cell carcinomas using immunohistochemical methods in this study to understand its function. Ten cases each of clinically and histopathologically confirmed oral lichen planus and oral squamous cell carcinoma were included in this study. The stain distribution in each OLP and OSCC cases were observed in the basement membrane. Immunohistochemical expression of laminin 5 gamma 2 shows a continuous line of staining in the basement membrane in 8 cases of OLP. In oral squamous cell carcinoma most cases (5/10) showed a discontinuous basement membrane immunohistochemical staining pattern. In accordance to this study, laminin expression is more strongly associated with the malignant potential of premalignant lesions such as OLP, and its total absence indicates a poorly differentiated carcinoma.

Keywords: Oral lichen planus,Oral squamous cell carcinoma, Laminin , Basement membrane

1. INTRODUCTION

Oral lichen planus (OLP) is a prevalent chronic inflammatory disease that primarily affects middle-aged women[1]. The possible malignant character of oral lichen planus has been the subject of extensive debate and discussion.There is a substantial chance that OLP lesions will acquire a cancerization field and undergo malignant transformation, according to several case reports and controlled studies[2]. The disintegration of the basement membrane has been associated to the migration of cancer cells in oral potentially malignant disorders and oral cancers, suggesting that it is a significant indicator of malignancy[3]. Laminin 5 γ2 is a fundamental component of epithelial basement membrane which has been proposed to be the biochemical counterpart of the anchoring filaments that attach basal keratinocytes to the basement membrane[4].

To gain insight into the function of laminin 5 in both malignant and potentially malignant diseases, the expression of laminin 5 in oral lichen planus and oral squamous cell carcinomas (OSCC)was investigated using the immunohistochemical approach.

2. MATERIALS AND METHODS

Immunohistochemical procedure

Ten cases each of clinically and histopathologically confirmed oral lichen planus and oral squamous cell carcinoma selected from the archives of Department of Oral Pathology and Microbiology, from 2018 to 2020.Paraffin sections 3µm thick were prepared, deparaffinized in xylene and rehydrated in graded series of alcohol. Endogenous peroxidase activity was blocked by immersing the sections in 3% peroxidase and the antigen was retrieved by using a pressure cooker with the sections in EDTA buffer (PH 8). The slides were incubated at room temperature for 60 min with the primary antibody laminin 5 gamma 2.The poly horseradish peroxidase reagent and DAB (3,3-Diaminobenzidine) formed the basis of the antibody detection method.Finally the sections were counterstained with Mayer's hematoxylin.
Routine haematoxylin and eosin staining was employed for the morphological observation.

Assessment of staining

A brown-colored final product was the indication of positive immunoreactivity. As internal positive control, the basement membranes of the muscles, blood vessels, nerves, and epithelium have been used.
The stain distribution in each OLP and OSCC cases were observed in the basement membrane.

3. RESULT

Histopathological examination of haematoxylin and eosin stained sections of the OLP samples revealed a band-like lymphocytic infiltrate in the subepithelial connective tissue as well as liquefactive degradation of the basal cell layer(Figure 1). According to WHO modification of the Broder's grading system[5], five of the ten OSCC cases were moderately differentiated three were well differentiated(Figure 2), and two were poorly differentiated(Figure 3).

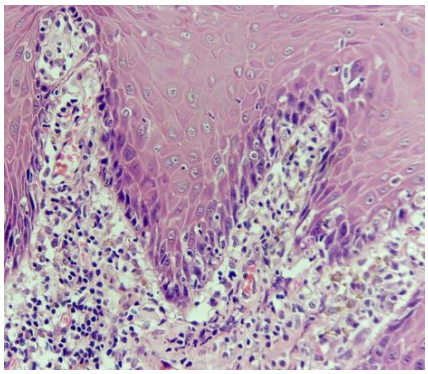

Figure 1 : Haematoxylin and eosin stained sections of the OLP (40x)

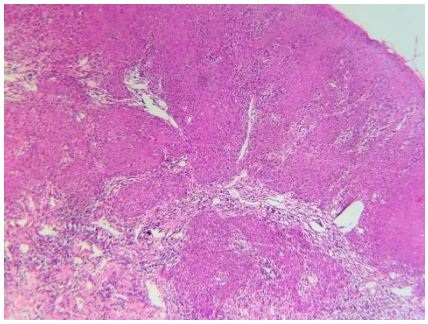

Figure 2 :Haematoxylin and eosin stained sections of WDSCC (10x)

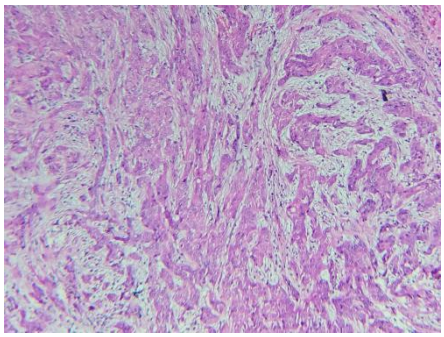

Figure 3 :Haematoxylin and eosin stained sections of PDSCC (40x)

Immunohistochemical expression of laminin 5 gamma 2 shows a continuous line of staining in the basement membrane in 8 cases of OLP (Figure 4),while irregular staining pattern was seen in 2 cases. (Table 1 and Figure 5).

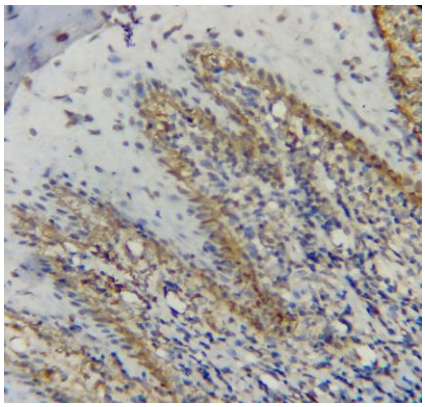

Figure 4: Continuous basement membrane staining in OLP (IHC,40x)

Parameter	Level	Count
Continuity around basement membrane	Positive	8
	Negative	2

Table 1: IHC expression of laminin 5 gamma 2 in OLP cases

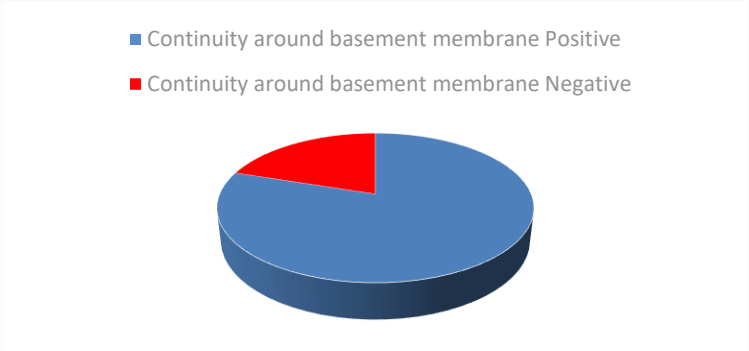

Figure 5: Pie diagram of continuity around basement membrane in OLP cases

In oral squamous cell carcinoma most cases (5/10) showed a discontinuous basement membrane immunohistochemical staining pattern. All cases of well differentiated squamous cell carcinoma (WDSCC) showed continuous basement membrane staining (Figure 6), moderately differentiated squamous cell carcinoma (MDSCC) showed two positive and three negative cases. All cases of poorly differentiated squamous cell carcinoma (PDSCC) revealed discontinous staining pattern of basement membrane with intracytoplasmic staining (Figure 7). Table 2 and Figure 8 are tabular and diagramatic representation of positive and negative cases of different grades of OSCC.

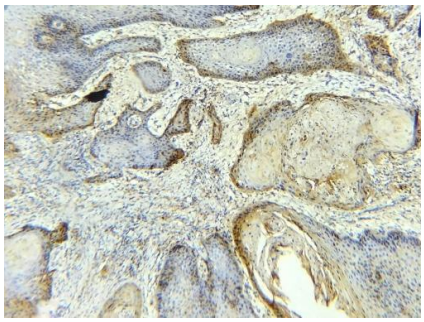

Figure 6 : Continuous basement membrane staining in WDSCC(IHC,10x)

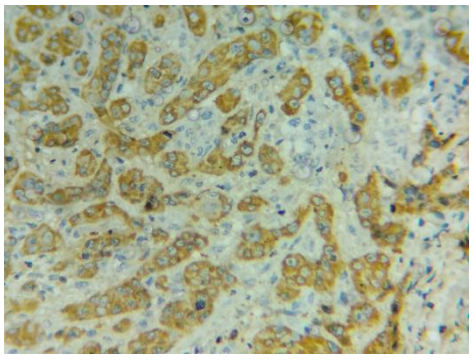

Figure 7: Intracytoplasmic staining without basement membrane staining in PDSCC(IHC,40x)

Parameter	WDSCC		MDSCC		PDSCC	
	Positive	Negative	Positive	Negative	Positive	Negative
Continuity around basement membrane	3	0	2	3	0	2

Table 2 : IHC expression of laminin 5 gamma 2 in OSCC cases

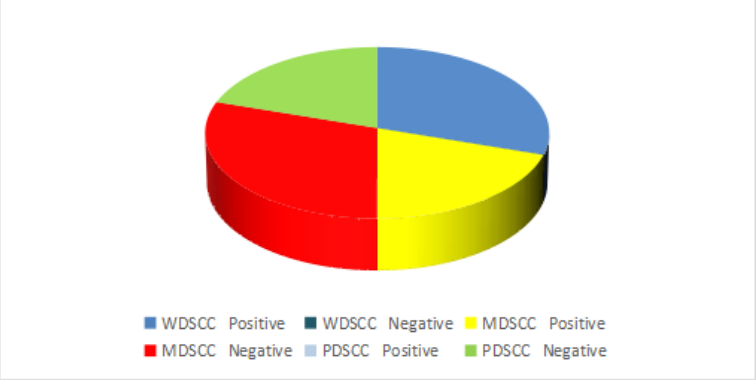

Figure 8: Pie diagram of continuity around basement membrane in OSCC cases.

4. DISCUSSION

A significant part of the basement membrane in the majority of adult tissues is laminin-5 , a heterotrimer made up of three chains (α3, β3, and γ2). One of the chains, laminin 5γ2, is often produced as a monomer in malignant tumors, making it a hallmark of invasive tumors[6].

The basement membrane serves as a support structure and barrier against neoplastic cells. It additionally serves a role in signaling during carcinogenesis by keeping carcinogenesis-modulating molecules, growth factors, and cytokines, which are activated when the membrane is breached. Laminin breakdown reveals carcinogenesis-inducing regions, crucial for neoplastic cell survival.

OLP often results in alterations to the epithelial basement membrane, such as breakages, branches, or duplications. Weaknesses at the epithelial-connective tissue interface brought on by basement membrane degeneration may lead to the creation of histological clefts.

Assessment of immunoreaction of basement membrane laminin can be helpful to rule out disruption of basement membrane and malignant transformation in OLP.

T Kainulainen et al.[7] reported immunoreaction to the laminin-5 was observed as a thin, continuous, delicate line in the basement membrane region in lichen planus , although in certain lichen planus cases, there were slight distinctions in the continuity and thickness.This investigation also revealed that the basement membrane was continuous in 8 out of 10 cases. The majority of the laminin-positive OLP samples in the buccal mucosa showed expressive discontinuous staining, according to Akama et al. [8]. This suggests that the pathogenesis of OLP and its progression to OSCC may be influenced by a altered epithelial basement membrane. In contrast, the majority of the laminin-positive samples in our investigation displayed continuous staining, which implies that cases are less likely to develop malignant alterations.

Higher grades of carcinomas were associated with frequent breaks of basement membrane continuity and greater intracytoplasmic staining, based on OSCC cases. Jiang et al. [9] and Harada et al. [10], who observed reduced basement membrane laminin expression in metastasized cancers, further supported this conclusion.The expression of laminin markings in WDSCC and subsequently less invasive tumors was strikingly similar to that of normal epithelium. All three of the WDSCC cases in the study displayed consistency in laminin expression. This result implies that laminin could still be secreted by tumor cells and cells close to the basal membrane.On the other hand, PDSCC revealed that all cases lost basement membrane continuity and had elevated intracytoplasmic staining. Similar result was obtained in a study conducted by de Almeida Reis SR et al[11].

According to this study, laminin expression is more strongly linked to premalignant lesions like OLP having the potential to become malignant, and its complete absence denotes a poorly differentiated carcinoma.

In accordance with this study, the malignant potential of premalignant lesions and differentiation of oral squamous cell carcinomas were probably determined by the expression of laminin, which shows basement membrane continuity.

5. CONCLUSION

Laminin expression can be associated with the progression of an invasive neoplastic cell phenotype and an optimal environment for tumor invasion. Therefore, we conclude that discontinuity of basement membrane laminin in OLP and OSCC should be considered a negative prognostic indicator of premalignant and malignant cases. To ascertain the clinical prognostic relevance of this finding, additional research should be conducted, including follow-up with patients diagnosed with oral lichen planus and oral squamous cell carcinoma.

6. REFERENCES

1. Au, J., Patel, D., & Campbell, J. H. (2013). Oral Lichen Planus. Oral and Maxillofacial Surgery Clinics of North America, 25(1), 93–100.
2. Gandolfo, S., Richiardi, L., Carrozzo, M., Broccoletti, R., Carbone, M., Pagano, M., … Merletti, F. (2004). Risk of oral squamous cell carcinoma in 402 patients with oral lichen planus: A follow-up study in an Italian population. Oral Oncology, 40(1), 77–83.
3. de Almeida Reis, S. R., Provedel de Souza, L. F., Ferreira de Souza, V., de Goes Silva, L. D., & Dos Santos, J. N. (2007). Expression of basement membrane laminin in oral squamous cell carcinomas. Brazilian Journal of Otorhinolaryngology, 73(6), 768–774.
4. Rousselle P, Lunstrum GP, Keene DR and Burgeson RE (1991) Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 114: 567–576 AC Broders. Squamous cell epithelioma of the lip. JAMA 1920;74:656–664.
5. Hamasaki H, Koga K, Aoki M, Hamasaki M, Koshikawa N, Seiki M, Iwasaki H, Nakayama J, Nabeshima K. Expression of laminin 5-γ2 chain in cutaneous squamous cell carcinoma and its role in tumour invasion. British journal of cancer. 2011 Sep;105(6):824-32.
6. T Kainulainen , H Autio-Harmainen, A Oikarinen, S Salo, K Tryggvason, T Salo. Altered distribution and synthesis of laminin-5 (kalinin) in oral lichen planus, epithelial dysplasias and squamous cell carcinomas.Br J Dermatol. 1997 Mar;136(3):331-6.
7. Akama MS, Teixeira LR, Innocentini LM, Gallo CD, Pinheiro TN, Ribeiro-Silva A, Motta AC. Laminin-332 expression in oral lichen planus: Preliminary results of a cross-sectional study. Oral Diseases. 2021 May 1;27(4).
8. Jiang D, Wilson DF, Smith PS, Pierce AM, Wiebkim OW. Distribuition of basal lamina IV collagen and laminin in normal rat tongue mucosa and experimental oral carcinoma: ultraestructural imunolocalization and imunogold quantitation. Euro J Cancer B Oral Oncol 1994:237- 43.
9. Harada T, Shinohara M, Nakamura S, Oka M. An immunohistochemical study of the extracellular matrix in oral squamous cell carcinoma and its association with invasive and metastatic potential. Virchows Arch 1994:257-66.

10. de Almeida Reis SR, de Souza LF, de Souza VF, de Góes Silva LD, Dos Santos JN. Expression of basement membrane laminin in oral squamous cell carcinomas. Brazilian Journal of Otorhinolaryngology. 2007 Nov 1;73(6):768-74.