Research Article

Immunohistochemical Expression of PTCH1 and Laminin in Oral Hyperplastic, Premalignant, Squamous Cell Carcinoma and Recurrence Lesions Samples

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Abstract

Objective: Laminin is a basal membrane glycoprotein that showed progressive loss of continuity from dysplasia to invasive carcinoma. The membranous receptor Patched (PTCH1) promotes the nuclear translocation and activation of the Gli family proteins. The dysregulation of hedgehog signaling reported in various cancers. This study aims to assess the immunohistochemical expression of PTCH1 and laminin in different groups of lesions of oral hyperplastic, premalignant, oral squamous cell carcinoma (OSCC) and recurrence cases.

Methods: This study involved 35 paraffin blocks of 4 oral hyperplastic, 11 premalignant, 15 OSCC and five recurrences OSCC cases collected from Sulaimani Histopathological Centers. Prepared tissue sections were stained immunohistochemically for both PTCH1 and laminin antibodies and scored. Chi-square correlations used and the $p < 0.05$ considered as statistically significant.

Results: PTCH1 showed expression in all oral hyperplastic lesions. While 81.8% of oral premalignant lesions demonstrated basal and parabasal distribution with high mixed localization (72.7%), lastly 93.3% of OSCC showed positive expression and mainly found within score 2(46.6%). No significant relations detected between oral hyperplastic and premalignant lesions regarding the expression pattern, localization and intensity as p-values were 0.77, 0.09 and 0.38 respectively. Lastly, the relations between OSCC and recurrent cases to both the expression and localization parameters were non-significant, as p-values were 0.15 and 0.09, respectively. Laminin showed continuous expression at the basement membrane of the normal oral mucosa, while only (50%) of the cases revealed such expression in oral hyperplastic lesions. The oral premalignant lesions expressed 54.5% of a discontinuous pattern. The relation between the oral hyperplastic and premalignant lesions in response to laminin expression was non-significant (P=.21). A significant relation found in laminin expression between OSCC and recurrence samples (p= 0.02). Finally, a significant correlation found between PTCH1 localization and laminin expression in oral premalignant lesions (p = 0.03).

Conclusions: The PTCH1 overexpression in all of the studied groups of lesions might give an impression of the active role of this biomarker in the progression toward malignancy. Laminin defragmentation, which started from dysplastic lesions extending to OSCC, could emphasize the role of this marker from the early precancerous stage. Furthermore, the combined PTCH1 mixed localization with discontinuous laminin expression might have a significant role in the progression of dysplastic lesions toward cancers.

Keywords: *PTCH1, Laminin, Oral premalignant lesions, OSCC.*

Submitted: March 18, 2019, Accepted: April 23, 2019, Published: June 1, 2019.

Cite this article as: Hamaamin KA, Hussein FA, Mohammad DN. Immunohistochemical Expression of PTCH1 and Laminin in Oral Hyperplastic, Premalignant, Squamous Cell Carcinoma and Recurrence Lesions Samples. Sulaimani Dent J. 2019;6(1):1-12.

DOI: https://doi.org/10.17656/sdj.10084

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Published by College of Dentistry, University of Sulaimani

Introduction

Oral premalignant lesions, known as potentially malignant disorders, are a group of diseases, which should be diagnosed in the early stage. Clinically, these diseases may sometimes simulate each other. Thus, the final diagnosis should confirm by biopsy. Although there are various etiological factors, the etiology of almost all of these diseases are not fully understood. Early diagnosis is essential and can be lifesaving as neglected lesions may progress to severe dysplasia and even become carcinoma in situ and squamous cell carcinoma[1].

Oral squamous cell carcinoma (OSCC) is characterized by malignant epithelial cells invasion that has squamous differentiation and identified by the formation of keratin and the presence of intercellular bridges[2]. It can occur as a primary lesion in any of the oral sites. However, most oral cancers detected in the tongue and buccal mucosa[3].OSCC considered among the 10th common cancers of the world[4]. They appeared initially as potentially malignant lesions of leukoplakia, erythroplakia, and oral submucous fibrosis[5].

During malignant transformation, normal cells experience the accumulation of several genetic and epigenetic alterations[6]. So, the transition of the normal epithelium to invasive cancer is a progressive process that is characterized by proliferation, angiogenesis, local invasion and eventually distant metastasis[7].

The hedgehog (Hh) pathway plays a critical role in normal embryonic development. It has a vital role in adult tissue maintenance, renewal, and regeneration. PTCH1 receptor activation on the cell membrane may initiate a series of cellular responses that range from survival, proliferation, cell fate specification, and differentiation[8]. Alteration in the hedgehog signaling pathway plays a role in malignant transformation among a subset of carcinomas, including lung, esophageal and pancreatic cancers[9, 10,11]. Wang et al.[12] showed PTCH1 overexpression in OSCC, which had a significant role in tumor growth, lymphatic metastasis, tumor recurrence, and patient's prognosis.

Laminin is a large heterotrimeric extracellular glycoprotein which is composed of α, β, and γ subunits. It is a component of the basement membrane and characterized by distinct domains with different structures and functions[13]. It is involved in cell adhesion, migration, proteolytic activity, cell proliferation, and metastatic growth[14, 15]. Tumor cells bind to laminin receptors on the basal membrane and are subsequently stimulated to produce metalloproteinase, which begins fragmentation and degradation of the membrane[16,17].

There was a tendency for discontinuous distribution of laminin from epithelial hyperplasia to epithelial dysplasia, with an increase in the discontinuity accompanying the increase in dysplastic grades[18]. The laminin expression decreased with high aggressiveness and low differentiation grade of OSCC[15,19,20]. This study detects the expression of both PTCH1 and laminin in the transition from normal oral mucosa to premalignant lesions reaching OSCC and recurrent cases, then relate the expression of these two markers in both oral premalignant and OSCC.

Materials and methods

This retrospective study conducted in Sulaimani Governorate during the period from December 2017 to November 2018. This study approved by the College of Dentistry Ethical Committee at the University of Sulaimani. The samples included four gingival oral hyperplastic tissues, 11 oral premalignant lesions (4 erythroleukoplakia, three lichen planus, three leukoplakia and 1 actinic cheilitis), of which (9 mild grade and two moderate grade) cases, 15 OSCC, and five recurrences OSCC cases that collected from Sulaimani Teaching Hospital and Shorish Hospital. The slides were prepared and stained in the pathology lab of College of the Dentistry/University of Sulaimani. Three serial five µm tissue sections were cut from each block and mounted, one stained with Hematoxylin and Eosin stain (H&E) for conforming histopathological features of lesions and grading of oral premalignant lesions and OSCC. The other two sections stained immunohistochemically for the two antibodies. The slides put in the oven (60ºC) for 6 hrs. Sections were deparaffinized by xylene, rehydrated by ethanol (100 %, 90%, and 70%). Antigen retrieval reached by boiling tissue sections in citrate buffer (pH of 6) for 15 min at 95ºC, then sections cooled at room temperature for 15 min and washed with PBS. The excess phosphate buffered removed gently, and the sections wiped around by gauze pad. Hydrogen peroxidase added to the sections at 37ºC for 10 min to block endogenous peroxidase activity and then washed twice with PBS. Protein block applied to the sections and incubated for 10 min to block nonspecific background staining, and then washed once with PBS. Primary antibodies applied to the sections; which were, Anti laminin (polyclonal Rabbit, dilution: 1:150) and PTCH1 (polyclonal Rabbit, dilution 1:150) (Abcam) and incubated for 45 min at 37ºC, then sections washed four times with PBS. Sections incubated with complement for 10 min and then washed twice by PBS. Sections incubated with conjugate for 15 min and then washed with PBS. Sections stained with DAB chromogen and incubated for 5 min in a dark field, then washed with PBS. Sections counterstained with hematoxylin for 20 sec; then the

slides washed with distilled water gently for 1 min. The sections then dehydrated in graded ethanol (70%, 90%,100%), then they put twice in xylene for 5 min each. Lastly, slides mounted with DPX and examined under a light microscope.

Normal oral mucosa served as a positive control for laminin[16], while for PTCH1 esophageal carcinoma acted as a positive control[21]. While the negative controls were done by omitting the primary antibodies and using the diluents alone. All sections were examined by two observers independently. For PTCH1 evaluation, Image J software for windows applied, and the immunostained cells calculated from 5 high spot fields pictures taken from a light microscope (at 400X), then they counted by a grid of the software. In oral hyperplastic and premalignant lesions, the expression pattern recorded as (basal, basal and parabasal, and full thickness). Cellular localizations were (nuclear, cytoplasmic, and mixed). Finally, the intensity was (faint, moderate, and strong). In OSCC and recurrence cases, the immunoscoring of (0, 0.5, 1, 1.5, 2, 3) used by multiplying intensity (0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining) by proportion of positively stained cells (0=<10%; 0.5=10-30%; 1>30%). Cellular localizations recorded as (nuclear, cytoplasmic, and mixed)[21]. The laminin staining evaluated at 400X magnification along the basal membrane. In oral hyperplastic and premalignant lesions; the staining classified as continuous; when the brown line remain along the epithelial and conjunctive border, or discontinuous; when there was a fragmentation line, and finally absent[19]. While in OSCC and recurrence cases, laminin expression evaluated semiquantitatively as follows:

Score 0: Continuous linear staining (no basement membrane (BM) defects).
Score 1: Loss of staining in less than 10% of the tumor-stromal interface per tumor cell nest (minor BM defects).
Score 2: Loss of staining in less than 50% of the tumor-stromal interface per tumor cell nest (moderate BM defects).
Score 3: Loss of staining in more than 50% of the tumor-stromal interface per tumor cell nest (BM defected to a large extent)[22].

Statistical analysis

Performed by the SPSS program (version 21). The data presented in tabular forms showing the frequency and distribution of different variables among the different groups of lesions. Chi-square tests used to compare the categorical data between the four different groups. P-value of 0.05 was used as a cut off point for the significance of statistical tests.

Results

PTCH1 expressed weakly in the basal layer of the normal oral mucosa (Figure 1, A). 75% of the oral hyperplastic samples revealed basal, and parabasal expression (Figure 1 B), with only one case (25%) showed full-thickness expression with mixed localization (Figure 1 C) and (Table 1).

All oral premalignant lesions had PTCH1 expression (100%). The expression was basal and parabasal in 9 dysplastic cases (7 mild and two moderate) (Figure 1, D), while two mild dysplastic cases showed full-thickness expression (Figure 1 E). Three cases had nuclear localization (27.2%), with high mixed localization (72.7%) (Figure 1, D-E) and (Table 1). No significant relations found between oral hyperplastic and premalignant lesions in response to the expression pattern, localization and intensity of PTCH1 as p values were 0.77, 0.09 and 0.38, respectively (Table 1).

PTCH1 showed positive expression in 14/15cases (93.3%) of OSCC. No case was with score 0.5, while the high percentage detected in score 2 (46.7%) (Table 2). Ten cases of OSCC were within the mixed localization (Table 2, and Figure 2, A), while the cytoplasmic localization found in four cases (Figure 2, B). In recurrent OSCC, 40% of cases equally reported in score 0 and score 2 (Table 2). The nuclear localization detected in 60 %, while mixed localization presented in 40% (Table 2 and Figure 2, C-D). The correlation between OSCC and recurrent cases in response to both the expression and localization of PTCH1 revealed non-significant relations; as p values were 0.15 and 0.09, respectively (Table 3).

Laminin showed a continuous linear pattern of expression at the BM of the normal oral mucosa (Figure 3, A). While in oral hyperplastic lesions, laminin appeared continuous at BM of 2 cases (50%) (Figure 3, B, and Table 4). In oral premalignant lesions, laminin had a discontinuous pattern in 6 cases (Figure 3, C), while 4 cases showed absent expression of laminin at BM (Figure 3, D). Finally, one case had a continuous expression (Figure 3, E, and Table 4). No significant relation was found in the pattern of expression of laminin in between the oral hyperplastic and premalignant lesions as p-value was 0.21 (Table 4).

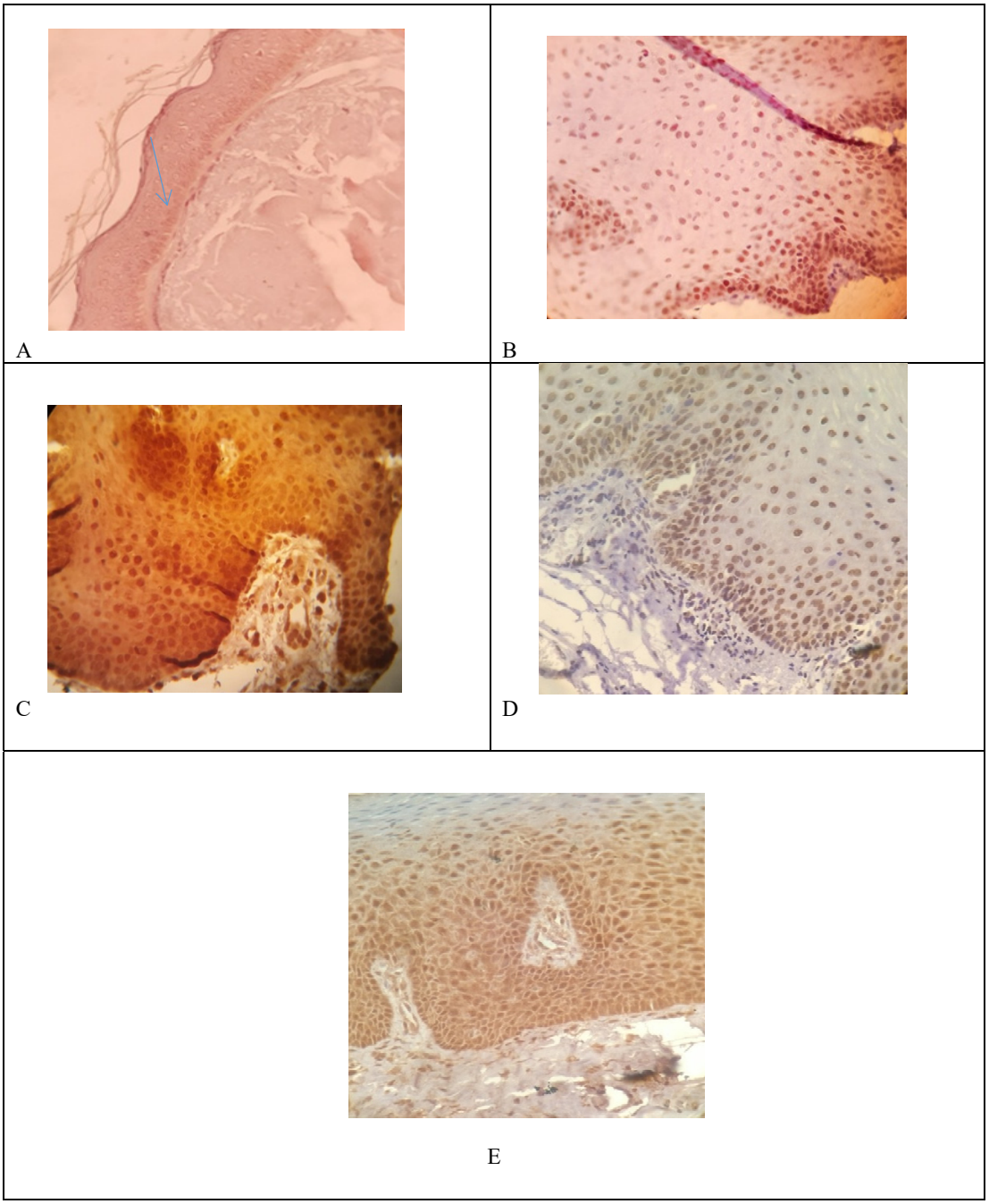


Figure 1: PTCH1 expression in normal oral mucosa, hyperplastic lesions and oral premalignant lesions(X400). A-Weak expression at the basal layer of the normal oral mucosa(blue arrow),B-Basal and parabasal expression in oral hyperplastic lesions, C-Full thickness expression with mixed localization in oral hyperplastic lesions, D-Basal and prarabasal with nuclear localization in premalignant lesions, E-Full thickness with mixed localization in premalignant lesions.

Table 1: PTCH1 expression pattern, localization and intensity in oral hyperplastic and premalignant lesions.

Oral hyperplastic lesions (4 cases)			Oral premalignant lesions (11 cases)		p values
		No. (%)	(9) Mild	(2) Moderate	
			No.(%)	No.(%)	
Expression	Basal	0	0	0	0.77
	Basal and parabasal	3(75)	7(77.7)	2(100)	
	Full thickness	1(25)	2(22.2)	0	
	Total	4(100)	9(100)	2(100)	
Localization	Nuclear	2 (50)	2(22.2)	1(50)	0.09
	Cytoplasmic	0	0	0	
	Mixed	2(50)	7(77.7)	1(50)	
	Total	4(100)	9(100)	2(100)	
Intensity	Faint	0	1(11.1)	1(50)	0.38
	Moderate	2(50)	6(66.6)	1(50)	
	Strong	2(50)	2(22.2)	0	
	Total	4(100)	9(100)	2(100)	

Table 2: PTCH1 expression and localization in OSCC and recurrence cases.

Immunoscoring							Localization in OSCC * and recurrence cases		
OSCC (15cases)	0	0.5	1	1.5	2	3	Nuclear	cytoplasmic	Mixed
	No.(%)	No.(%)	No.(%)	No(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
Well OSCC (7 cases)	0	0	1(14.2)	0	5(71.4)	1(14.2)	0	2(28.5)	5(71.4)
Moderate OSCC (7cases)	1(14.2)	0	1(14.2)	1(14.2)	2(28.5)	2(28.5)	0	2(28.5)	4(57.1)
Poor OSCC (1case)	0	0	0	0	0	1(100)	0	0	1(100)
Recurrence (5cases)	2(40.)	1(20)	0	0	2(40)	0	3(60)	0	2(40)

*One OSCC had no expression

Table 3: PTCH1 correlation in response to the expression pattern and localization between oral squamous cell carcinoma and recurrence cases.

		OSCC		Recurrence		p value
		No.	%	No.	%	
Expression of PTCH1	0	1	(6.7)	2	(40)	0.15
	0.5	0	(0.0)	1	(20)	
	1	2	(13.3)	0	(0)	
	1.5	1	(6.7)	0	(0)	
	2	7	(46.7)	2	(40)	
	3	4	(26.7)	0	(0)	
	Total	15	(100)	5	(100)	
Localization of PTCH1	Mixed	10	(66.7)	2	(40)	0.09
	Nuclear	0	(0)	3	(60)	
	Cytoplasmic	4	(26.7)	0	(0)	
	Total	14	(100)	5	(100)	

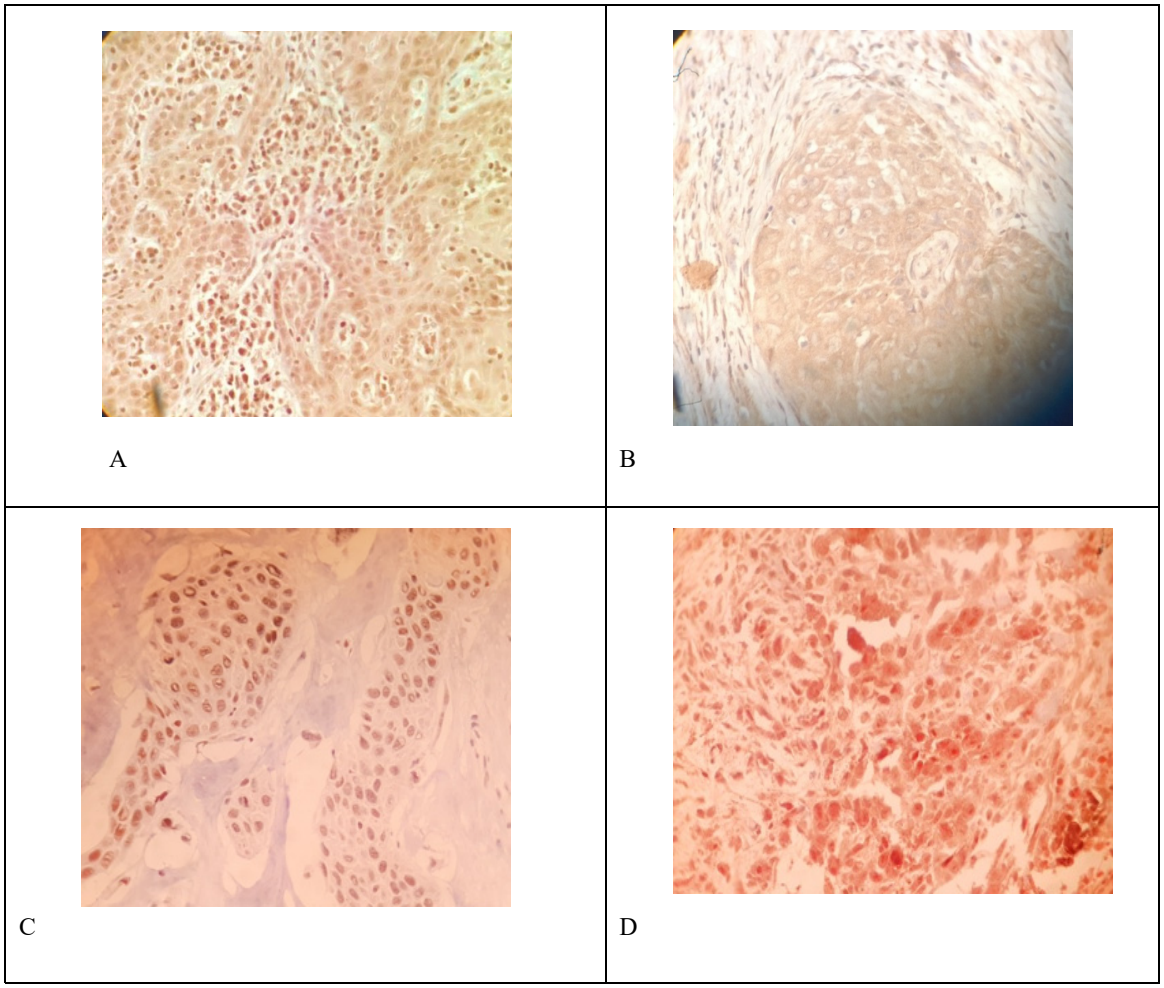
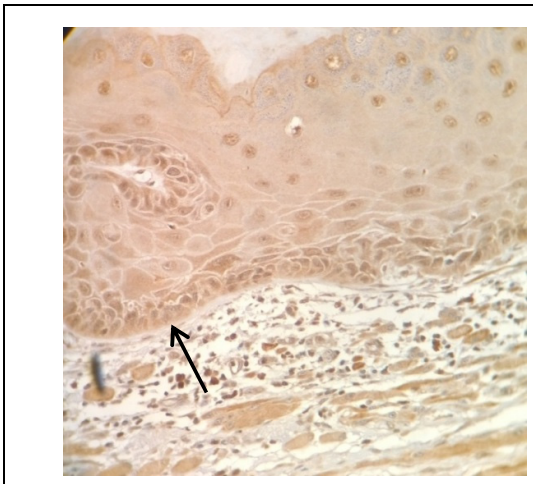


Figure 2: PTCH1 localization in OSCC and recurrent cases (X400): A-Mixed localization in OSCC, B- Cytoplasmic localization in OSCC, C- Nuclear localization in recurrent cases, D- Mixed localization in recurrent cases.



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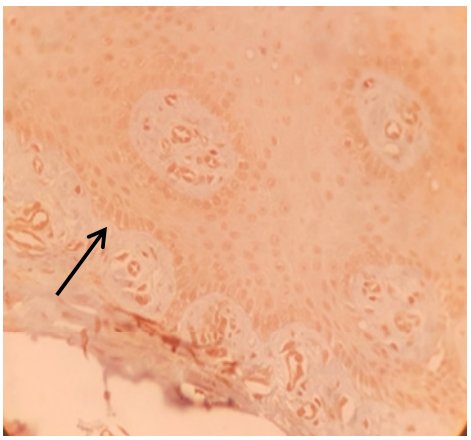

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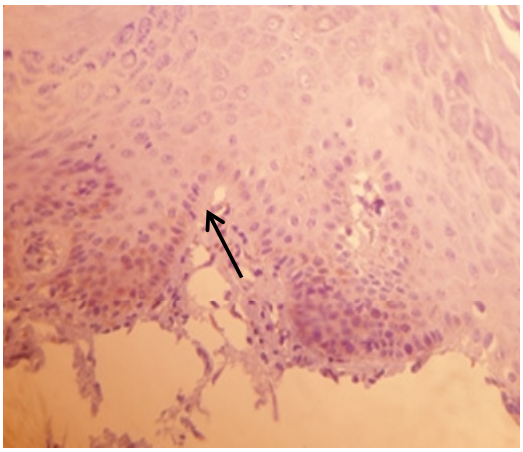

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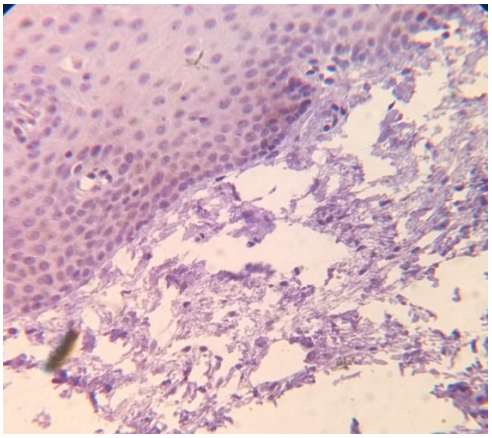

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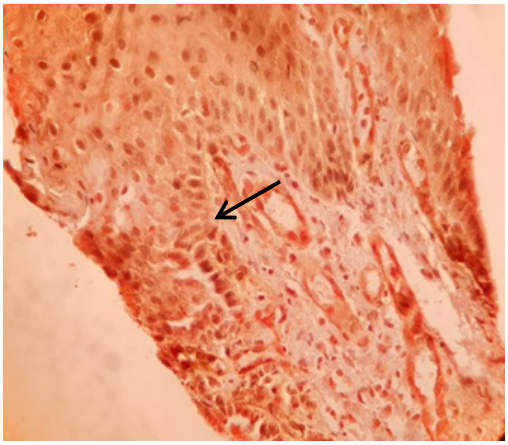

E

Figure 3: Laminin expression in normal oral mucosa, oral hyperplastic and premalignant lesions(X400):A-Continuous expression at the BM of normal oral mucosa (black arrow), B- Continuous expression at the BM of the oral hyperplastic lesions (black arrow),C- Discontinuous expression at the BM of the oral premalignant lesions (black arrow), D-Absent expression in oral premalignant lesions. E-Continuous expression at the BM of the oral premalignant lesions (black arrow).

Table 4: Laminin pattern and immunoscoring in oral hyperplastic, premalignant lesions, OSCC and recurrence cases.

Oral hyperplastic lesions (4cases)		Premalignant lesions (11 cases)			
		Mild (9 cases)	Moderate (2 cases)		p value
Expression pattern	No.(%)	No.(%)	No.(%)		
Absent	1(25)	3(33.3)	1(50)		
Continuous	2(50)	1(11.1)	0		0.21
Discontinuous	1(25)	5(55.5)	1(50)		
Total	4(100)	9(100)	2(100)		
OSCC and recurrence cases	Well OSCC	Moderate OSCC	Poor OSCC	Recurrence cases	P value
Immunoscoring	No. (%)	No. (%)	No. (%)	No. (%)	
0	1(14.2)	1(14.2)	0	0	
1	2(28.5)	0	0	2(40)	*0.021
2	0	0	0	2(40)	
3	4(57.1)	6(85.7)	1(100)	1(20)	
Total	7(100)	7(100)	1(100)	5(100)	

*p value significant <0.05

In OSCC, 11/15(73.3%) were within score 3 (BM defects to a large extent) (Table 4, and Figure 4, A). Only 2 cases found in score 0 (no BM defects) (Fig 4, B). Finally, two well-differentiated OSCC revealed score 1 (minor BM defects) (Figure 4, C, and Table 4). In recurrence squamous cell carcinoma, score three detected in 20% of all cases (Figure 4, D, and Table 4). The relation of laminin expression between the OSCC and recurrence cases reached the level of significance as The p-value was 0.02 (Table 4). In oral premalignant lesions, the relation between PTCH1 localization and laminin pattern of expression reached the level of significance as the p-value was 0.03, while no significant association found between PTCH1 expression and laminin pattern as p-value was 0.36. In OSCC laminin scoring did not reveal significant relations in response to both PTCH1 expression and localization, as p values were 0.72 and 0.53 respectively.

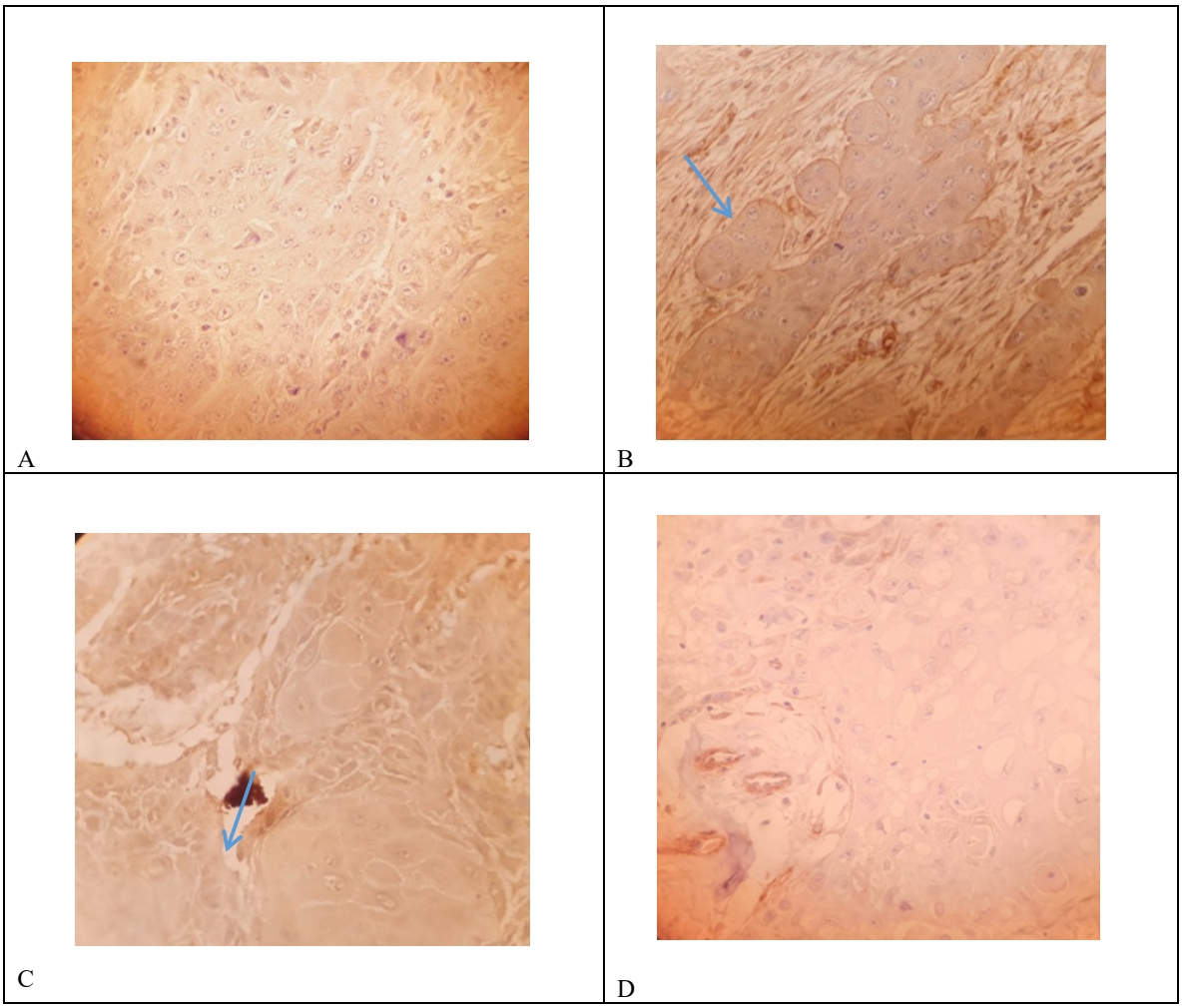

Figure 4: Laminin expression in OSCC and recurrent cases (X400): A- Discontinuous pattern (Score 3) in OSCC, B- Continuous pattern (no BM defect) (score 0) in OSCC (blue arrow), C-Minor BM defect (score 1) in OSCC islands (blue arrow), D- Major defect of BM (score 3) in recurrent cases.

Discussion

This study reported the weak expression of PTCH1 in normal oral mucosa; this was in agreement with a study done by Wang *et al.*[12]. All the oral hyperplastic cases showed positive expression (100%). This finding explained by the types of the samples as all of them were from the gingiva that might be affected by a masticatory force and irritating factors of plaque accumulation.

All oral premalignant lesions had PTCH1 expression; this overexpression was similar to the study done by Dias *et al.*.[23]. In this study, most cases showed basal and parabasal expression (81.8%). This finding was in disagreement with the study done by Yang *et al.*.[21], as they found 21% of dysplastic esophageal lesions with this pattern of expression. This study revealed PTCH1 expression in both mild and moderate dysplastic lesions (9 milds, two moderates). While Yang *et al.*[21] found in their study that PTCH1 expression was only seen in severe dysplastic lesions, this study showed high mixed localization of PTCH1 in premalignant lesions (72.7%). Such expression might detect the different stages of activation of this marker that could have a role in the progression from the dysplastic lesions to cancers.

PTCH1 detected in (93.3%) of OSCC. While Wang *et al.*[12] detected this marker in 47.5% of OSCC cases, this variation in the expression could be due to the difference in sample size. The most prominent score in

this study was score two, which composed 46.6%. This was in agreement with the study done by Wang *et al*.[12], as they found score 2 to be 47.5%. Most cases of OSCC presented with mixed localization (71.4%), which was in disagreement with the finding of Leovic *et al*..[24], as they reported high membranous localization. The finding of this study could be explained by signal activation that facilitates the transcription of certain genes that contribute to cell migration, angiogenesis and then promote tumor progression. The correlation between OSCC and recurrence cases did not reach the level of significance; a similar finding observed by Wang *et al*.[12] while Srinath *et al.*[25] showed a highly significant relation (p=0.001) of sonic Hh protein expression in their cancerous samples compared with the dysplastic lesions.

In normal oral mucosa, laminin showed a continuous linear pattern of expression. This was in accordance with the study done by Garcia *et al*.[19]. While, in the oral hyperplastic lesions, laminin had a continuous expression in 50% of cases. Different results detected by other authors; as Firth and Reade[26] showed that laminin distribution was continuous in all of their oral hyperplastic samples, while Kannan *et al*.[27] reported laminin discontinuity in all of their oral hyperplastic cases. This difference reasoned to differences in sample size and type of lesions used in this study. In oral premalignant lesions, laminin revealed high discontinuous and absent distributions. Similarly, other studies demonstrated the discontinuous distribution of laminin in their premalignant samples[19,27,28]. The laminin expression with both discontinuous and absent pattern indicates the role of this marker in the early diagnosis of potentially malignant lesions and could predict the biological progression toward malignancy.

Laminin showed a high score of 3 (73.3%) in OSCC. This finding was in line with the results of Kobayashi *et al*.[22] and Garcia *et al.*[19], as they found a prominent major defect in their samples. However, it was in contrast with Mostafa *et al*.[29] and Shruthy *et al*.[13] studies in which a high percentage of their cases had continuous staining of laminin around the basement membrane. This major defect of laminin found in both moderately and poorly differentiated OSCC, which were 85.7% and 100% respectively. While Souza *et al*.[16] in their study detected 50% of poorly differentiated SCC were within the major defect. The finding of this study supports the fact that laminin fragmentation gradually is increased with the loss of differentiation of OSCC[19]. Continuous laminin expression was only seen in well and moderately differentiated OSCC (14.2% for each). The expression is less than that found by other researchers in well-differentiated OSCC, as they reported the continuous expression to be 90% and 52.6%[13,16]. This difference could be related to different numbers of evaluated islands in selected samples of OSCC and the sample size. 40% of recurrence OSCC cases recorded in both minor and moderate defects. Stoltzfus *et al*.[30] found that (75%) of their recurrence cases had prominent staining in their islands (≥50% staining). The prominent minor and moderate defects of laminin in recurrence OSCC might be related to the ability of this marker to facilitate the development of the second primary tumor and could be used as a predictive biomarker for aggressiveness of oral cancer and distant metastasis, as a significant relation of laminin scoring was found between oral squamous cell carcinoma and recurrence samples (p = 0.02) while Yellapurkar *et al*.[15] detected non-significant relation of laminin expression in OSCC in response to the pattern of invasion (p=0.71).

Finally, the relation between laminin pattern and PTCH1 localization in oral premalignant lesions reached the level of significance. Thus, the prominent discontinuous pattern of laminin and mixed localization of PTCH1 in these premalignant lesions might illustrate the combined role of these two markers in the initial invasion of basement membrane that could pave the way for further migration later on.

Conclusion

The PTCH1 plays a vital role in the progression toward malignancy and might be considered as a potential therapeutic target. The expression of laminin is dramatically decreased from oral hyperplastic lesions toward OSCC and could be a useful parameter to evaluate tumor histological differentiation and aggressiveness. Finally, the discontinuous laminin pattern with different PTCH1stage of activation started early from premalignant lesions.

References

1. Yardimci G, Kutlubay Z, Engin B, TuzunY. Precancerous lesions of oral mucosa. World J Clin Cases. 2014;16(12):866-72.

2. Rajendran R. Benign and malignant tumors of the oral cavity. In: Rajendran R, Sivapathasundharam B, editors. Shafer's Textbook of Oral Pathology. 6th ed. New Delhi-India: Reed, Elsevier; 2009.p.101.

3. Sarkis SA, Abdullah BH, Abdul Majeed BA, Talabani NG. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and

lymphangiogenesis. Head Neck Oncol. 2010;2(1):13.

4. Sharma P, Saxena S, Aggarwal P. Trends in the epidemiology of oral squamous cell carcinoma in Western UP. Indian J Dent Res. 2010;21(3):316-19.

5. Hernandez BY, Zhu X, Goodman MT, Gatewood R, Mendiola P, Quinata K, et al. Betel nut chewing, oral premalignant lesions, and the oral microbiome. PLoS ONE. 2017;12(2):e0172196.

6. Irani S. Pre-Cancerous Lesions in the Oral and Maxillofacial Region: A Literature Review with Special Focus on Etiopathogenesis. Iran J Pathol. 2016;11(4):303–22.

7. Tsantoulis PK, Kastrinakis NG, Tourvas AD, Laskaris G, Gorgoulis VG. Advances in the biology of oral cancer. Oral Oncol. 2007;43(6):523-34.

8. Wilson CW, Chuang PT. Mechanism and evolution of cytosolic Hedgehog signal transduction. Development. 2010;137(13):2079-94.

9. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature. 2003;422(6229):313-17.

10. Sims–Mourtada J, Izzo JG, Apisarnthanarax S, TehWu T, Malhotra U, Luthra R, et al. Hedgehog: an attribute to tumor regrowth after chemoradiotherapy and a target to improve radiation response. Clin Cancer Res. 2006;12(21):6565-72.

11. Onishi H, Katano M. Hedgehog signaling pathway as a new therapeutic target in pancreatic cancer. World J Gastroenterol. 2014;20(9):2335-42.

12. Wang YF, Chang CJ, Lin CP, Chang SY, Chu PY, Tai SK , et al. Expression of Hedgehoge signaling Molecules as a prognostic indicator of oral squamous cell carcinoma. Head neck. 2012;34(11):1556-61.

13. 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(2):185-9.

14. Engbring JA, Kleinman KH. The basement membrane matrix in malignancy. J Pathol. 2003;200(4):465-70.

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(2): 407-13.

16. Souza LF, Souza VF, Silva LD, Santos JN, Reis SR. Expression of basement membrane laminin in oral squamous cell carcinomas. Braz J Otolaryngol. 2007;73(6):768-74.

17. Kinoshita T, Nohata N, Hanazawa T, Kikkawa N, Yamamoto N, Yoshino H, et al. Tumour-suppressive micro-RNA-29s inhibit cancer cell migration and invasion by targeting laminin-integrin signalling in head and neck squamous cell carcinoma. Br J Cancer. 2013;109(10):2636-45.

18. Degen M, Natarajan E, Barron P, Widlund HR, Rheinwald JG. MAPK/Erk dependent translation factor hyper activation and dysregulated Laminin γ2 expression in oral dysplasia and squamous cell carcinoma. Am J Pathol. 2012;180(6):2462-78.

19. García SA, Abad-Hernández MM, Fonseca-Sánchez E, Julián -Gonzalez R, Galindo-Villardón P, Cruz-Hernández JJ, et al . E-cadherin, laminin and collagen IV expression in the evo-lution from dysplasia to oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2006;11(2):E100-5.

20. Abdel Rahman GA, El Bolok AH, Essa TE. Immunohistochemical expression of laminin-5γ2 in oral squamous cell carcinoma. Egypt Dent. J. 2015;61(3):3535-39.

21. Yang L, Wang LS, Chen XL, Gatalica Z, Qiu S, Liu Z, et al. Hedgehog signaling activation in the development of squamous cell carcinoma and adenocarcinoma of esophagus. Int J Biochem Mol Biol. 2012;3(1):46-57.

22. Kobayashi Y, Nakajima T, Saku T. Loss of basement membranes in the invading front oral squamous cell carcinoma with high potential of lymph node metastasis: An immunohistochemical study for laminin and type IV collagen. Pathol Int. 1995;45(5):327-34.

23. Dias RB, Valverde Lde F, Sales CB, Guimarães VS, Cabral MG, de Aquino Xavier FC, et al. Enhanced expression of hedgehog pathway protiens in oral epithelial dysplasia. Appl Immunohistochem Mol Morphol. 2016;24(8):595-602.

24. Leovic D, Sabol M, Ozretic P, Musani V, Car D, Marjanovic K, et al. Hh-Gli signaling pathway activity in oral and oropharyngeal squamous cell carcinoma. Head Neck. 2012;34(1):104-12.

25. Srinath S, Iyengar AR, Mysorekar V. Sonic hedgehog in oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol. 2016;20(3):377-83.

26. Firth NA, Reade PC. The prognosis of oral mucosal squamous cell carcinomas: a comparison of clinical and histopathological grading and of laminin and type IV collagen staining. Austr Dent J. 1996;41(2):83-6.

27. Kannan S, Balaram P, Chandran GJ, Pillai MR, Mathew B, Nalinakumari KR, et al . Alterations in expression of basement membrane proteins during tumor progression in oral mucosae. Histopathol. 1994;24(6):531-7.

28. Rani V, McCullough M, Chandu A. Assessment of laminin-5 in oral dysplasia and squamous cell carcinoma. J Oral Maxillofac Surg. 2013;71(11):1873-9.

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

30. Stoltzfus P, Högmo A, Lindholm J, Aspenblad U, Auer G, Munck-Wikland E. The gamma2 chain of laminin-5 as an indicator of increased risk for recurrence in T1 stage tongue cancer. Anticancer Res. 2004;24(5B):3109-14.