Perilesional sites of OSCC had altered P-cadherin expression

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Abstract

Background: Epithelial cells at surgical free margins are considered to be free of pathology at the morphological level. However, they might be associated with molecular changes when considering clonal expansion of pre-neoplastic cells in a particular tumor field; that subsequent genomic changes could drive them towards the malignant development.

Aim of Study: To evaluate the distribution and cellular localization of P-cad at the perilesional area of OSCC, in comparison with normal oral mucosa and correlate such expression to clinical parameters and histopathological grading of the primary lesion.

Materials and Methods: P-cad expression was assessed immunohistochemically in archival paraffin blocks of 20 oral mucosa adjacent to OSCC (perilesional sites) and 10 normal oral mucosa.Epithelial cell layers distribution and intracellular localization wererecorded.

Results: Normal oral mucosa revealed membranous basal and parabasal expression. All the perilesional samples showed positive results with 55% overexpression. The cytoplasmic expression alone or mixed with membranous localization was the predominant expression (18 out of 20 cases). Sex, age, and clinical presentations did not show any significant relations in response to P-cad distribution or intracellular localization. While P-cad intracellular localization was significantly correlated with the histopathological grading (P=0.046) and epithelial cell layers distribution (P=0.026). P-cad overexpression was seen in 81.8% in WDSCC (9/11) perilesional sites.

Conclusion: P-cadherin overexpression and shifting its intracellular localization from membranes to cytoplasmic and mixed at the perilesional site of oral cancer could be considered as a mark for revealing cancerization and lateral spreading.

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Introduction

The Early detection of premalignant changes in oral mucosa is a challenging event (1).The oral cavity is one of the predominant sites for developing of potential malignancies since it comes into direct contact with different carcinogenic agents. The squamous cell carcinoma is one of the most common malignancies developed with an average survival rate of about five years (2). OSCC arises as a result of several clinical changes in the affected epithelial tissues in conjunction with molecular and biochemical cellular alterations and associated fibrovascular stroma (3).

Surgical margins that are histopathologically free from tumor might be associated with either recurrences or second primary tumors, which could support the concept of field of cancerization (2), Furthermore, oral cancer does not develop as an isolated cellular phenomenon, but rather as an anaplastic tendency involving multiple foci of cells as a result of repeated carcinogenic attack and progresses at various rates within the entire field. (4, 5).

On the other hand, normal oral mucosa becomes continuously exposed to a variety of carcinogens, which subsequently lead to multiple genetic and epigenetic abnormalities. The initial genetic events might be associated with clonal expansion of pre-neoplastic daughter cells in a particular tumor field; later on, subsequent genomic changes could drive them towards the malignant development. So on similar genetic alterations in oral dysplastic, potentially malignant lesions, and fully established OSCC could be seen. (6, 5). The technological improvement and carefully designed studies with appropriate control tissue will enable identification of important molecular biomarkers in these genetically altered but histologically are normal looking cells (6). The significant behind this is its application through primary chemoprevention for normal tissues adjacent to tumors (peri-tumoral cancer fields) (7).

The term "Lateral cancerization" is subsequently used to indicate a lateral spread of tumors which is due to the progressive transformation of cells adjacent to a tumor, rather than the spread and destruction of the adjacent epithelium by pre-existing cancer cells (8). It is observed that normal looking cells near malignant cells are histologically abnormal and therefore are considered as a part of the transformed cells in a particular tumor field, and consequently are responsible for the occurrence of local tumor recurrences (9).

P-cadherin (P-cad) is cell adhesion molecule, which is only expressed in the basal and suprabasal cell layers of the normal oral epithelium. During tumor progression, P-cad expression increases in the initial stage of tumor growth, whereas a reduced membranous and or an enhanced cytoplasmic expression of P-cad is observed at the invasion front of oral squamous cell carcinoma (OSCC) (10). Furthermore, studies revealed that the expression of an inappropriate cadherin in epithelial cells is yet another way that tumor cells can alter their adhesive function (11).

Understanding the role of P-cad at the peri-tumoral site of oral SCC is a prerequisite for improving the patient prognosis since dissociation of cell-cell adhesion is the first step in epithelial invasion (12). This study aimed to identify P-cad expression, distribution, and localization at the perilesional margins of surgically removed OSCC samples.

Materials and methods

This study included 20 archival paraffin blocks of OSCC with perilesional area collected from different histopathological centers. Ten blocks of normal oral mucosa were used as positive controls. The study was approved by both the scientific and ethical committee of the School of Dentistry.

Serial 4µm sections were cut, one section was stained with hematoxylin and eosin to identify the perilesional area of the lesion and histopathological grading of OSCC. The other section was mounted on positively charged slide and stained immunohistochemistry for P-cad identification. Sections were deparaffinized and rehydrated; then antigen retrieval solutions were applied (Citrate buffer, pH 6) by using the pressure cooker (for15 min). Endogenous peroxidase activity was blocked by incubating the sections with 1% H2O2 in PBS (for 10 min). To prevent non-specific binding sections were incubated with 1.5% blocking serum (for 1 hour). Then sections were incubated with the primary mouse monoclonal antibody of P-cad (U.S Biological; diluted at 1:20) for three-quarters of an hour at 37°C in a humid chamber. Biotinylated secondary antibody was applied for 30 min at 37°C, and then detection was performed by using the avidin-biotin-peroxidase technique for 30 min at 37°C. The reaction was visualized by incubation with diaminobenzidine for 10 minutes, and then sections were counterstained with hematoxylin. Negative control slides were obtained by omitting the primary antibody.

The sections were examined blindly and evaluated for P-cad expression, in surface epithelium at perilesional sites of OSCC, according to the parameters mentioned

below. The resulted findings were related to the clinicopathological feature of each case.

P-cad expression and distribution within epithelial layers, which was compared with that found in the normal oral mucosa (13; 14; 15). It was categorized to

be within single basal layer or extend to and assessed as follow (11);

Unchanged expression; strong immunoreactivity confined to basal cells' layer.

An increases expression: strong and faint positive expression extend to multiple epithelial layers

Decreased expression; faint cytoplasmic staining at the basal cells' layer.

P-cad cellular localization was grouped into three categories including; membranous, cytoplasmic and mix (both membranous and cytoplasmic).

SPSS statistical software was used to estimate Chi-square test. Probabilities of less than 0.05 were accepted as significant.

Results

This study showed that normal oral mucosa had a basal and a parabasal P-cad expression (Figure 1).

All the perilesional samples showed positive expression with different distribution (Figure 2). An increases expression (multiple layers distribution) was found in 45% with strong staining (Figure 3A), and in another case (10%) with faint staining. While decreased expression (faint cytoplasmic restricted to the basal layer), was seen in 25% (Figure 3 B). The remaining cases (20%) showed unchanged expression at basal cells' layer. Nevertheless, they had different intracellular localization (Figure 3 C and D, Figure 3, Table 1).

There were no differences in P-cad distribution in epithelial cells' layers and intracellular localization related to the sex or the age group of the patients or the clinical presentation of the lesions. Variation of carcinoma histopathological grading did not reach a significant level of difference (P=0.196) in P-cad distribution expression, while its intracellular localization was significantly related to the grading (P=0.046) as well as to the distribution of expression (P=0.026). The results showed nine patients out of 15 with well differentiated SCC had overexpression, and the cytoplasmic expression alone or mixed with membranous localization was the predominant expression (18 out of 20 cases) seen in peri-lesional sites of all histopathological grades except the single case of poorly differentiate OSCC which showed basal membranous distribution (Table -1 and 2, and figure 3 D).

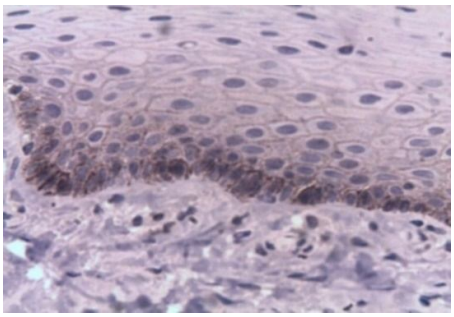

Figure 1- P-cad expression, distribution, and localization in normal oral mucosa showed positive membranous expression with basal and parabasal distribution.

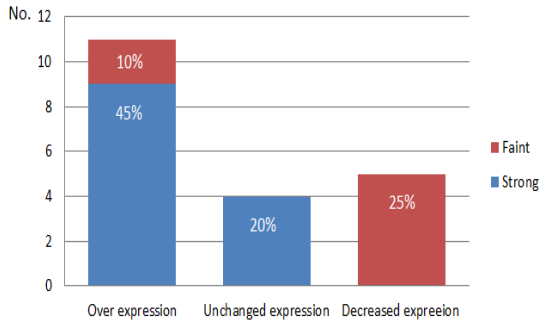

Figure 2- The distribution of P-cadherin expression in perilesional sites of OSCC

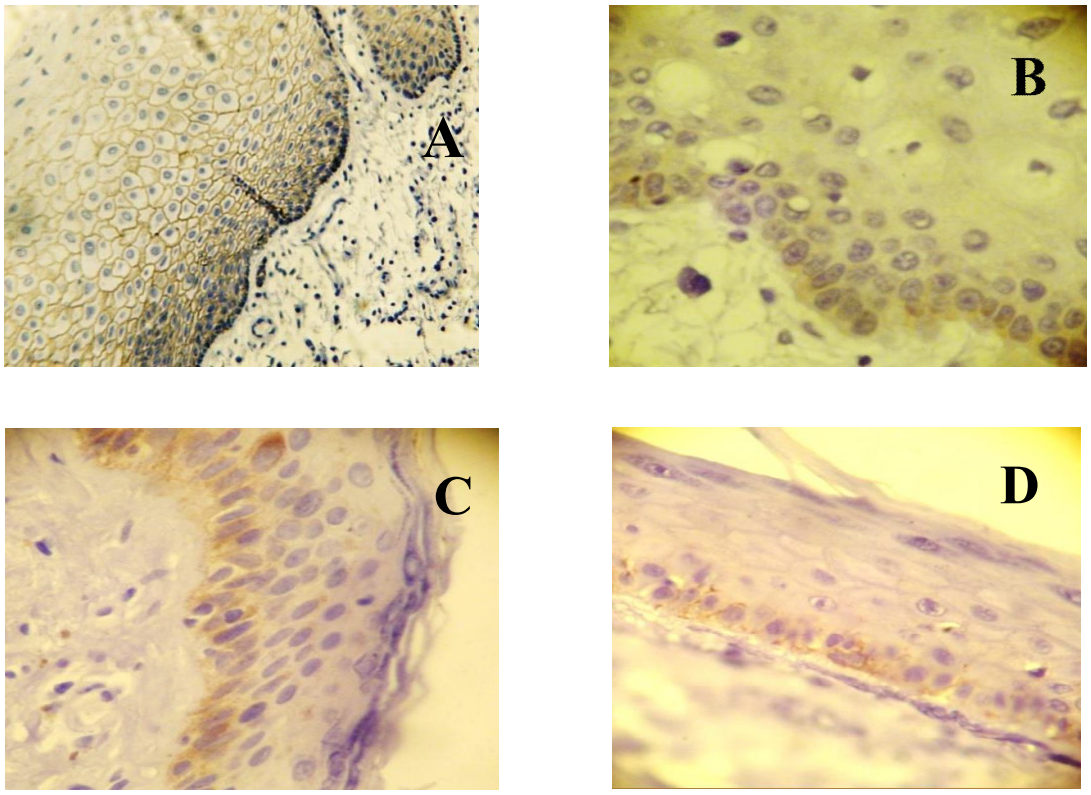

Figure 3- P-cad expression, distribution, and localization in perilesional surgical margins of OSCC showed A- a membranous expression of multiple epithelial cell layers. B–faint cytoplasmic expression with basal distribution. C-mixed membranous and cytoplasmic expression limited to the basal layer. D-membranous expression limited to the basal layer.

Table-1: The P-cadherin expression and distribution in the perilesional area of OSCC regarding the sex, age, clinical presentation, site and grading of the primary lesion.

Categories	Sub-groups	Total		Expression						Chi-Square Tests
				Unchanged		Over*		Under		
		No.	%	No.	%	No.	%	No.	%	
Sex	Male	14	70	3	75	7	63.6	4	80	.78
	Female	6	30	1	25	4	36.4	1	20	
Age	≤ 50	8	40	2	50	4	36.4	2	40	.893
	› 50	12	60	2	50	7	63.6	3	60	
Clinical appearance	Ulcer	14	70	2	50	8	72.7	4	80	.66
	Mass	5	25	2	50	2	18.2	1	20	
	White	1	5	0	0	1	9.1	0		
Perilesional site	Tongue	5	25			2	18.2	3	60	.336

	Lower lip	6	30	2	50	3	27.2	1	20	
	Floor of mouth	1	5			1	9.1			
	Cheek	4	20	2	50	2	18.2			
	Maxilla and palate	2	10			2	18.2			
	Mandible	1	5			1	9.1			
	Alveolar mucosa	1	5					1	20	
Grading	Well	15	75	3	75	9	81.8	3	60	.196
	Moderate	4	20	0		2	18.2	2	40	
	poor	1	5	1	25					
Localization	Membranous	2	10	1	25	1	9.1			.026
	cytoplasmic	8	40	1	25	2	18.2	5	100	
	mixed	10	50	2	50	8	72.7			

Table-2: The P-cadherin intracellular localization in perilesional OSCC sites regarding the sex, age, clinical presentation, site and grading of the primary lesion.

Categories	**Sub-groups**	**Total**	**Intracellular localization**						**Chi-Square Tests**
			Membranous		Cytoplasmic		Mixed		
		No.	No.	%	No.	%	No.	%	
Sex	Male	14	2	100	5	62.5	7	70	.585
	Female	6	0		3	37.5	3	30	
Age	≤ 50	8			4	50	4	40	.435
	› 50	12	2	100	4	50	6	60	
Clinical appearance	Ulcer	14	2		6	75	6	60	.731
	Mass	5			2	25	3	30	
	White	1					1	10	
Perilesional site	Tongue	5			3	37.5	2	20	.047
	Lower lip	6	1	50	4	50	1	10	
	Floor of mouth	1	1	50					
	Cheek	4					4	40	
	Maxilla and palate	2					2	20	
	Mandible	1					1	10	
	Alveolar mucosa	1			1	12.5			
Grading	Well	15	1	50	6	75	8	80	.046
	Moderate	4			2	25	2	20	
	poor	1	1	25	0				
Expression	Unchanged	4	1	50	1	12.5	2	20	.026
	Over	11	1	50	2	25	8	80	
	Under	5			5	62.5			

Discussion

Evidence supports that a tumor could be surrounded by a mucosal field of genetically altered cells. Thus biopsies from the histopathologically normal mucosa adjacent to the tumor or at the surgical margins could share some or even all genetic markers with the tumor, indicating that both have arisen from a common cell clone (9). Later on the accumulation of these genetic changes forms the basis for the progression from a normal to a cancer cell, Finally clonal divergence leads to the development of one or more second tumors within a contiguous field of pre-neoplastic cells (16).

Kato et al. (2006) (17) indicated that p16 methylation is detected in healthy peritumoral tissue of OSCC, and considered it as an early event in carcinogenesis. Later on, Ruesga et al., (2007) (18) reported a higher level of methylation in histologically healthy samples from patients previously treated for OSCC and considered to increase the risk for predisposition to tumor recurrence. Furthermore, 33.3% of E-cad methylation was found in the perilesional area of OSCC, and 12.5% of these areas showed the same degree of methylation as the cancerous lesion. (19, 20).

Extensive researches had been done considering the altered expression of P-cadherin in OSCC (14, 11, 10), however, till now there is no study that evaluates its expression in the perilesional area of OSCC.

This marker reported positive expression in all perilesional areas of OSCC samples. Eleven cases (55%) showed overexpression distribution. This aberrant expression is in consistent with the concept of "field of cancerization," and opens a clue for identification of molecular signatures in the genetically transformed but histologically normal-looking epithelial cells (peri-tumoral cancer field). It might help in preventing transformation of pre-malignant sites into carcinoma (21). It is worth to mention that OSCC also had been reported to have a similar percentage of P-cad overexpression (11, 22). Therefore, the primary lesion and the perilesional site could be developed from a single clone and share common genetic alterations; such relationship might drift progenitor cells and result in cancerization. (9, 2). Although age might play a role concerning the accumulation of multiple genetic alterations (23), the present study indicated that neither the sex nor the age of the patient with OSCC had significant relations to P-cad distribution or localization in the mucosa surrounding the lesion. A large number of ulcerated OSCC had P-cad overexpression (8 out of 14) with a cytoplasmic multi-layers distribution, again with no significant relation. Furthermore, lip, tongue, and check were the frequent sites reported in the peri-lesional sample in descending manner. However, peri-lesional mucosa from SCC of the tongue was more frequently with decreased P-cad expression. One cannot draw an explanation unless having an idea about the variation of P-cad expression of the primary lesions at these sites. But differences in the type of epithelial and prognosis of OSCC in these sites may play a role. Beside that intracellular localization is significantly differed about different sites of the oral cavity. Never the less, decrease P-cad expression is an indicator of cancerous transformation and a greater chance for invasion (24). Possibly larger sample size may better declare the above points.

P-cad showed overexpression in perilesional sites of WDSCC (9/15, 60%) in a similar to that reported in the primary lesion (22,14). While the peri-tumoral margin of a single case of PDSCC showed unchanged basal membranous expression like that of normal oral mucosa. However, the relation did not reach a significant level. On the other hand, the intracellular localization revealed significant relation to the histopathological grading. Likewise, both basal and multilayer P-cad expression had significantly prominent mixed intracellular localization (membranous and cytoplasmic) which may be considered as a significant indicator, since a similar alteration reported in the primary OSCC is indicating

an event before the invasion (25). Thus this expression might have a role in the mode and path of lateral spreading and invasion along the surrounding mucosa that had no cytologic or morphologic abnormalities.

New genomic and proteomic profiling techniques need to be applied for early detection of any alteration.

Conclusion

Histopathologically normal mucosa adjacent to the tumor or at the surgical margins showed altered P-cadherin expression, sharing that with the primary lesion. They showed multilayer distribution and cytoplasmic localization. The importance of such alteration may be considered in the early carcinogenesis and lateral spreading.

References

1.YardimciG,Kutlubay Z, Engin B, TuzunY.Precancerous lesions of oral mucosa. World J Clin Cases. 2014 ; 2(12): 866-872.

2. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts.Oncol Rev .2014; 8:244.

3. MessadiVD. Diagnostic aids for detection of oral precancerous conditions. Inte J Oral Sci. 2013; 5:59–65.

4. Ashwini KB, Sharada P, Hema NK, Chitra MS. Oral field cancerization: An update of evidences. J NTR Univ Health Sci.2015;4 (3):141-4.

5. Wisniewski JDM, Ma T and Schneider A. Advances in the chemopreventive targeting of oral carcinogenesis.Curr Oral Health Rep. 2015 ; 2:63–72.

6. Jaiswal G, Jaiswal S, Kumar R, Sharma A. Field cancerization: concept and clinical implications in head and neck squamous cell carcinoma.JExpTherOncol. 2013;10(3):209-14.

7. Tsao AS, Kim ES, Hong WK. Chemoprevention of cancer. CA Cancer J Clin. 2004; 54(3):150-80.

8. Dakubo DG, Jakupciak PJ, Birch-Machin AM, and Parr LR. Clinical implications and utility of field cancerization. Cancer Cell Int. 2007; 7: 2.

9. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res .2003; 63:1727–1730.

10. Bauer K, Gosau M, Reinders J, Oefner P, Reichert ET and Bauer R. Presenilin 1/γ-secretase modulates P-cadherin processing and influences cell adhesion in oral squamous cell carcinoma cell lines. Carcinogenesis .2013;34 (11): 2622-2628.

11. PyoSW,HashimotoM,Kim YS, Kim CH, Lee SH, Johnson KR, Wheelock M J and Park JU. Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: correlation with the clinicopathologic features and patient outcome. J CraniomaxillofacSurg.2007; 35:1-9.

12. Hashimoto T, Soeno Y, Maeda G, Taya Y, Aoba T, Nasu M, KawashiriS,Imai K. progression of oral squamous cell carcinoma accompanied with reduced E-cadherin expression but not cadherin switch. PLoS ONE. 2012; 7(10): e47899.

13. Lo Muzio L, Pannone G, Mignogna MD, Staibano S, Mariggiò MA, Rubini C, Procaccini M, Dolci M, Bufo P, De Rosa G and Piattelli A. P-cadherin expression predicts clinical outcome in oral squamous cell carcinomas. HistolHistopathol. 2004; 19:1089-99.

14. Lo Muzio L, Campisi G, Farina A, Rubini C, Pannone G, Serpico R,.LainoG, De Lillo A and Carinci F. P-cadherin expression and survival rate in oral squamous cell carcinoma: an immunohistochemical study. BMC Cancer. 2005; 5:63.

15.Bauer R ,Dowejko D, Driemel O, Boßerhoff AK and Reichert TE .Truncated P-cadherin is produced in oral squamous cell carcinoma .FEBS J .2008; 275: 4198–4210.

16.Chai H and Brown ER. Field effect in cancer–An update. Ann Clin Lab Sci. 2009 ; 39 (4) :331-337.

17.Kato K, Hara A, Kuno T, et al: Aberrant promoter hypermethylation of p16 and MGMT genes in oral squamous cell carcinoma and the surrounding normal mucosa. J Cancer Res ClinOncol. 2006;132: 735-743.

18.Ruesga MT, Acha-Sagredo A, Rodríguez MJ, et al: p16INK4a promoter hypermethylation in oral scrapings of oral squamous cell carcinoma riskpatients. Cancer Lett. 2007; 250: 140-145.

19. Yeh KT, Shih MC, Lin TH, et al: The correlation between CpGmethylation on promoter and protein expression of E-cadherin in oral squamous cellcarcinoma. Anticancer Res .2002; 22: 3971-3976.

20. Díez-Pérez R, Campo-Trapero J , Cano-Sánchez J., López-DuránM,.Gonzalez-Moles M.A,. Bascones-Ilundain J and Bascones-Martinez A.Methylation in oral cancer and pre-cancerous lesions (Review). Oncol Rep. 2011; 25: 1203-1209.

21. Aparna M, Shenai P, Chatra L, Veena KM, Rao KP, Prabhu VR, ShahinKA.Fieldcancerization: A review. Arch Med Health Sci.2013 ; 1 (2):136-9.

22. Mohammad ND,. Garib TB, Khudeir HH & Abdul–Majeed AB. Immunohistochemical expression of P-cadherin and cortactin in oral squamous cell carcinomas. Sulaimani Dent J. 2015;2(1):8-14.

23. Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara Rey JM, García-García A. Genetic and molecular alterations associated with oral squamous cell cancer (Review).Oncol Rep. 2009 ;22(6):1277-82.

24. Feller LL, Khammissa RR, Kramer BB and Lemmer JJ .Oral squamous cell carcinoma in relation to field precancerisation: pathobiology.Cancer Cell Inte. 2013; 13:31.

25. Williams H.K, Sanders D.S A, Jankowski JA Z, Landini G. and Brown A.M S. Expression of cadherins and catenins in oral epithelial dysplasia and squamous cell carcinoma. J. Oral Pathol. 1998; 27:308-317.