Immunohistochemical expression of P-cadherin and cortactin in oral squamous cell carcinomas

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Abstract

Objectives: to assess the expression and pattern distribution of P-cadherin and cortactin in OSCC, and to relate such expression to the histopathological grading.

Materials and Methods: An immunohistochemical staining for P-cadherin and cortactin was performed on paraffin blocks of 40 oral squamous cell carcinoma and five normal mucosa. Fisher's exact test and Spearman's rank-order correlation were applied for analysis. $P < 0.05$ was considered statistically significant.

Results: P-cadherin was either aberrantly cytoplasmic re-localized or lost in 45% of cases. 58.6% of positive cases were in grade I. Focal heterogeneous pattern was the commonest pattern (27.5%) and related to the degree of cell differentiation. The expression percentage was reported mainly in score 1 and 2 with no differences among histopathological grades (P=0.778). On the other hand, 70% of oral SCC had cytoplasmic cortactin expression. The majority of cases (85.7%) expressed diffuse pattern including all positive grade II and III cases. Nevertheless, statistical analysis did not reach a significant level (P= 0.722). Furthermore, no significant correlation was found between P-cadherin and cortactin expression.

Conclusions: Oral SCC had P-cadherin under-expression (focal) and cortactin over-expression (diffuse). The P-cadherin pattern distribution rather than expression percentage was related to the degree of cell differentiation. The expressions of these molecules were unrelated to each other.

Keywords: *oral squamous cell carcinoma, P-cadherin, cortactin.*

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Introduction:

Oral cancer represents the sixth common form of the malignant disease (it is third in incidence in developing countries and ranks about eighth in developed countries). Oral squamous cell carcinoma (OSCC) accounting for 95% of all oral malignant lesions [(1)]. Despite significant advances in surgical techniques and introduction of adjuvant treatment modalities, the overall prognosis of OSCC has not improved [(2-4)]. It characterized by a local invasion with a tendency for spreading to cervical lymph nodes. The ability of malignant cells to invade surrounding tissues is one of the major hallmarks that distinguish them from normal cells [(5)].

Invasion and metastasis represent complex, multistep processes. They include; loss of cell adhesion, cytoskeletal rearrangements, migration and dissolution of cell at the basement membrane, intravasation, survival (in blood vessels), extravasation (at a distant site), and growth of migrating cells in the distant place. The process also stimulates neo-angiogenesis [(6)]. During invasive and metastatic progression, the first step characterized by increased cell motility and invasiveness. It has been hypothesized that these processes may related to epithelial-mesenchymal transition [(6,7)].

During malignancy progression, the regulation and expression of cell-cell adhesion molecules (like cadherins) play a fundamental role. In most invasive tumors, E-cadherin is down-regulated, and N-cadherin is de novo expressed [(4)]. P-cadherin (P-cad) plays a role in the maintenance of the epithelial phenotype. It is concerned, together with E-cad, in the final stage of tumor progression in epidermal carcinogenesis. It has become more evident that P-cad contributes to the oncogenesis of many tumors. Only a few studies evaluate its immunohistochemical (IHC) expression in OSCC. They show that decreased or truncation P-cad expression is associated with low differentiation. Such

the abnormal expression is linked with poorer overall and disease-free survival rate [8-11].

On the other hand, cortactin is an F-actin binding protein. It stabilizes F-actin networks and promotes actin polymerization. Over-expression of cortactin reported in several human cancers. Its expression is more frequent in higher grade tumors and invasive pattern, as it stimulates cell migration, invasion, and metastasis [12].

There are many studies dealing with the molecular changes in oral SCC concerning the proliferation, apoptosis, angiogenesis and growth factors; however, few studies are published concerning invasion and metastasis. Therefore, the aims of the present study are; first to assess the expression and pattern distribution of P-cad and cortactin, in order to predict the molecular changes regarding both adhesion loss and cytoskeletal rearrangement in OSCC. Second to relate such expression to the histopathological grading.

Materials and Methods:

The study involved forty blocks of OSCCs cases collected from Baghdad and Sulaimani histopathological centers. The age, sex, site of the lesions and type of surgery were registered from case sheets. (10 incisional biopsies, 11 excisional biopsies and 19 treated cases with radical neck dissection) (inadequate data for TNM staging for incisional biopsies). Five blocks of mucoceal containing normal oral mucosa were obtained from Oral Pathology Department at Sulaimani University. The ethical committee of the Faculty of Medical Sciences approved the work.

Serial 4µm sections were cut and mounted on positively charged slides; one section stained with hematoxylin and eosin to confirm histopathological grading (Broder' classification) and the remaining were subjected to IHC staining. Sections were deparaffinized and rehydrated, immersed in antigen retrieval solutions (Citra buffer, pH 6) and pretreated in the pressure cooker (15 min). Endogenous peroxidase activity was blocked by 0.1% H2O2 (10 min), and non-specific binding of antibodies was blocked by 1.5% blocking serum (1 hr). Then sections incubated with the primary monoclonal antibodies (anti P-cad; Biological, US; diluted at 1:20, 45 min at 37°C, and anti-cortactin; Santa Cruz, diluted at 1:50, 1hr at 37°C). Sections were treated with biotinylated secondary antibody (30 min at 37°C) and were allowed to be detected by using the avidin-biotin-peroxidase technique. The reactions were visualized by diaminobenzidine for 20 min then counterstained with hematoxylin. Negative control sections were treated with phosphate buffer saline instead of the primary antibody.

From each section, five hot spot fields were examined (from either the lateral or deeper sites of the lesion whenever possible). An eyepiece (square grid) attached to a light microscope ocular and epithelial cells (minimum of 1000 positive cells) evaluated for each case at a magnification of X400. The collective percentage of positive cells among all sections examined was determined. All slides were assessed blindly and independently by two pathologists. The reliability of inter-observer agreement for the readings was evaluated by Cohen's test, and the yielding values were higher than 0.70 in almost all instances. Then the average of readings for each case was obtained. After one month, the slides were reassessed by the same pathologists, in the same way, and the obtained scores were statistically not significantly differed from first reading.

When evaluating the expression of P cad protein, intense cell surfaces membranous staining in most cells was interpreted as positive. Diffuse cytoplasmic staining was considered negative [13]. The antibodies were evaluated semi-quantitatively as follows: P-cad percentage expression: score1<25%, score2= 26-50%, score3= 51-75% and score4 > 75% (table -1). While P-cad distribution pattern alone analyzed under X100 and scored as follow: score1=absent, score2 focal heterogenous, score3= reduced homogenous, and score4= strong homogenous [14]. Cortactin cytoplasmic expression: score0= negative, score1 =5-20% weak focal staining, score2= 20-50% strong focal staining, score3 >50% strong diffuse staining [15].

R software (http://www.r-project.org/) was used to estimate Fisher's exact test and SPSS software to calculate Spearman's rank-order correlation. Probabilities of less than 0.05 accepted as significant.

Table 1: The semi quantitative scoring systems used for evaluating P-cad distribution pattern, P-cad expression percentage and cortactin expression.

P cadherin [Lyakhovitsky et al., 2004].				Cortactin [Greer et al.,2007]	
Expression pattern		Expression	Percentage	Score	Percentage and pattern
Score 1	Absent	**Score 1**	1 -25%	**0**	Negative
Score 2	Focal heterogenous	**Score 2**	26-50 %	**1**	5-20 %focal cytoplasimic
Score 3	Reduced homogenous	**Score 3**	51-75%	**2**	20-50% focal cytoplasimic
Score 4	Strong homogenous	**Score 4**	>75%	**3**	>50 diffuse cytoplasimic

Results:

OSCC was observed in patients with a mean age of 55 ± 15.2 years. It is predominantly seen in males (65%), most commonly involving the tongue (35%) and presented as ulcer (70%). Twenty-nine cases (72.5%) were well differentiated (grade I), and only 3 cases (7.5%) were poor differentiated (grade III), table-2.

Normal oral epithelium showed positive membranous P-cad expression in the basal and parabasal layers (figure-1A) and negative cortactin expression, whereas vascular endothelial cells revealed intense positive cortactin expression (figure-1B).

Oral SCC showed 55% (n= 22) positive P-cad expression (figure-2 A) predominantly in grade I (58.6%). Focal heterogeneous pattern (figure-2D) was the predominant pattern (27.5%, table-3), while the expression percentage was high in both score 1 (40.9%) and in score2 (31.81%) with no significant differences among histopathological grades (P>0.05) (table-4).

On the other hand, 70% of oral SCC (n=28) had cytoplasmic cortactin expression and the majority (85.7%) expressed diffuse cytoplasmic pattern (table-5, figure 3 A-C). No case reported in score 1 and all positive grade II and III cases seen at score3. Nevertheless, statistical analysis did not reach a significant level (P= 0.722) (table-5). Furthermore, no significant correlation was found between P-cad and cortactin expression.

Table 2: Frequency distribution of the OSCC sample by clinical presentation and tumor site.

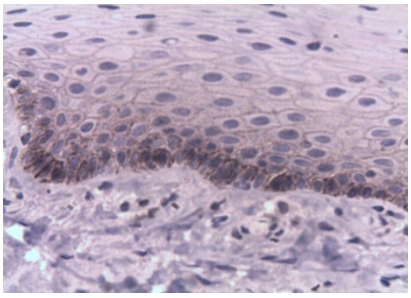
		No.	%
Sex	**Male**	26	65
	Female	14	35
Presentation	**Ulcer**	28	70
	Mass	7	17.5
	White lesion	5	12.5
Site	**Tongue**	14	35
	Lip	6	15
	Buccal mucosa	6	15
	Maxilla and hard palate	6	15
	Mandible	3	7.5
	Floor of mouth	2	5
	Alveolar mucosa	2	5
	Unknown	1	2.5
Histopathology	**Grade I**	29	72.5
	Grade II	8	20
	Grade III	3	7.5

Discussion:

Cancerous cells in oral SCC growth either had abnormal or lost P-cad expression in nearly half of the studied cases. The positive cases had predomenent focal distribuion that related to the degree of cell differentiation. Thus, P-cad pattern rather than P-cad expression percentage in oral SCC is more informative. On the other hand, cortactin scoring system expressed both of these items (pattern and percentage) simultaneously and accordingly oral SCC had a large percentage of aberrant cortactin expression with strong diffuse pattern especially in grade III. The expressions of these adhesion molecules were unrelated to each other.

P-cad is a protein homologous to E-cad. It mediates homophilic and homotypic adhesion between cells in contact, they are essential for initiating and maintaining cell-cell contact and cell polarity. In normal epidermis, P-cad is detected on surface of basal and suprabasal keratinocytes [9,11,16].When these cells migrate to the differentiated compartment, they down-regulate P-cad expression [9].

In this study, P-cad was detected in the cytoplasm of cancerous cells that are indicating an abnormal expression, and probably they did not share in cells adhesion. The importance of understanding the differential functional roles and biological implications between the extracellular and intracellular domains of P-cadherin have not emphasized earlier.



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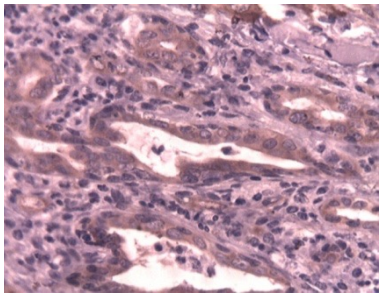


B

Figure 1: Normal oral epithelia; positive membranous basal and parabasal P-cad expression (A). Positive cytoplasmic cortactin expression in endothelial cells (B) (final magnification X400).

Table 3: Frequency and percentage distribution of P-cadherin expression pattern in OSCC in relation to histopathological grading.

Expression pattern			Total		Grade I		Grade II		Grade III		P value
			No.	%	No.	%	No.	%	No.	%	
Negative	Score 1	Absent	18	45	12	41.37	4	50	2	66.66	0.659
	Total positive		22	55	17	58.63	4	50	1	33.34	
Positive	Score 2	Focal heterogenous	11	27.5	9	31.03	2	25	0	0	
	Score 3	Reduced homogenous	7	17.5	6	20.68	1	12.5	0	0	0.469
	Score 4	Strong homogenous	4	10	2	6.89	1	12.5	1	33.33	

Table 4: Frequency and percentage distribution of 22 OSCC positive P-cadherin cases in different expression scoring and in relation to histopathological grading.

Score	Expression percentage	Total		Grade I		Grade II		Grade III		P value
		No	%	No.	%	No	%	No	%	
Score 1	1 -25%	9	40.9	7	41.17	2	50	0	0	
Score 2	26-50 %	7	31.81	6	35.29	1	25	0	0	0.778
Score 3	51-75%	5	22.72	3	17.64	1	25	1	100	
Score 4	>75%	1	4.545	1	5.88	0	0	0	0	

Table-5: Frequency and percentage distribution of cortactin expression in OSCC in relation to histopathological grading.

Percentage and pattern		Score	Total		Grade I		Grade II		Grade III		P value
			No	%	No.	%	No.	%	No	%	
Negative		0	12	30	9	31.03	3	37.5	0	0	
	Total positive		28	70	20	68.97	5	62.5	3	100	0.720
Positive	**5-20 %focal cytoplasmic**	1	0	0	0	0	0	0	0	0	
	20-50% focal cytoplasmic	2	4	14.3	4	20	0	0	0	0	0.722
	>50 diffuse cytoplasmic	3	24	85.7	16	80	5	100	3	100	

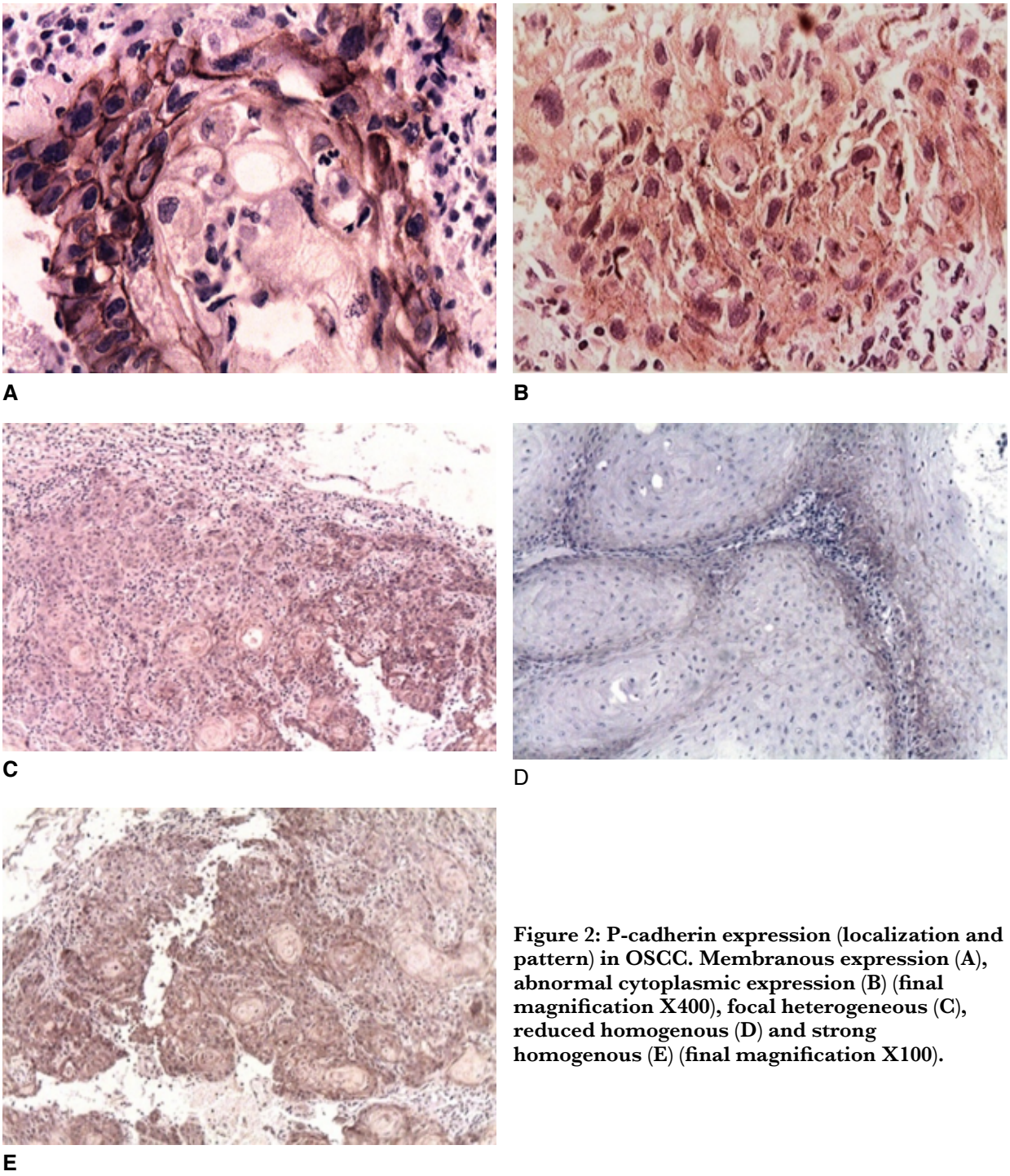
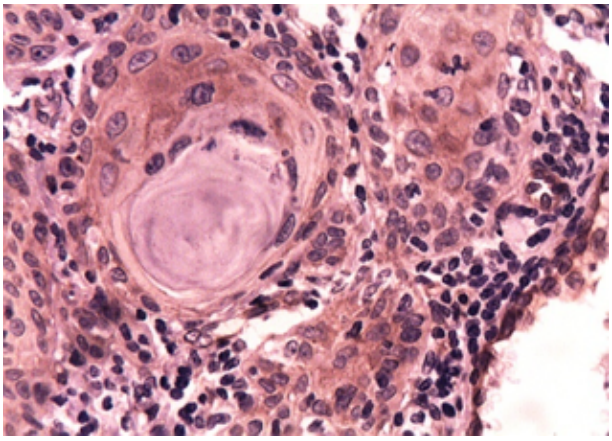


Figure 2: P-cadherin expression (localization and pattern) in OSCC. Membranous expression (A), abnormal cytoplasmic expression (B) (final magnification X400), focal heterogeneous (C), reduced homogenous (D) and strong homogenous (E) (final magnification X100).

The differences in the number and type of samples, the methods of immunohistochemical staining, criteria for analyzing histological grading and P-cad scoring make it difficult to compare different studies. In this work P-cad staining intensity did not consider, all modes of cell growth (islands, strand and single infiltrating cells) were included, and a cytoplasmic re-localization regarded as a negative expression. We reported 55% positivity that was higher than Williams et al. [17] findings (33%) and seemed to approximate Lo Muzio et al. [10] result (55.2%). But in fact, we observed higher positivity since they considered both membranous and cytoplasmic localization as a positive expression. In another study, Munoz-Guerra et al. [10] reported conserved P-cad expression in (42%) without demarcating the exact localization of stain interpretation. While Pyo et al. [18] considered suprabasal P-cad expression as increased expression (53.06 %), basal P-cad expression as unchanged (18.3%). They measured faint P-cad stained cancer cells as under-expressed (28.5%).

The type distribution of P-cad expression in OSCC was not studied before. In this study, half of the positive cases were focal heterogenous (11 out of 22). This expression identifies greater cell populations with



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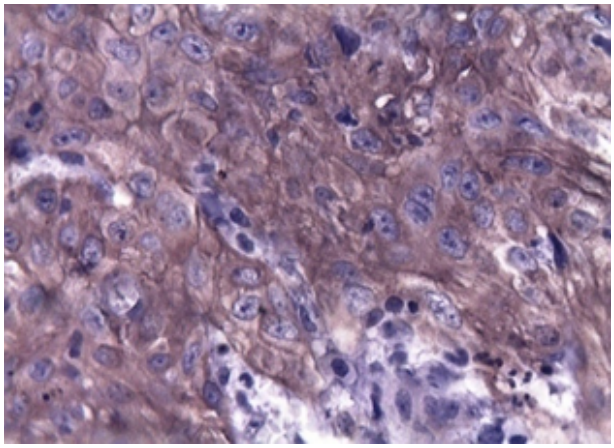

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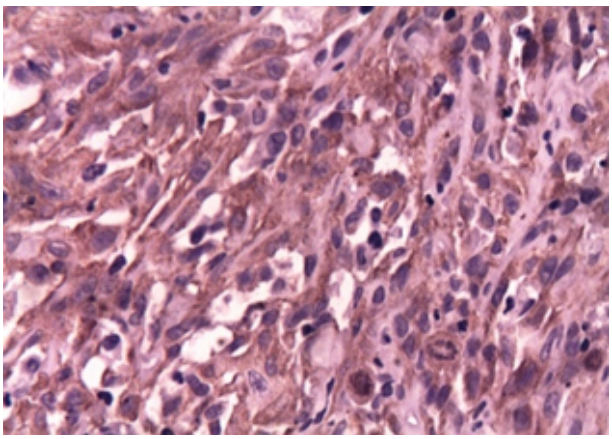

C

Figure 3: Cortactin expression in OSCC. More than 50% of cells showed diffuse cytoplasmic expression in grade I (A), grade II (B) and grade III (C) SCC (final magnification X400).

loss of adhesion surrounded by focal areas of the adhesive mass (proliferating islands). Since P-cad expression increases with dividing basal and suprabasal layers and decreases as epithelial cells differentiate [9]. In carcinomas growth, under favorable conditions E-cad reduce its expression to facilitate invasion. Whereas the tumor cells re-express E-cad and renew the intercellular adherence when the surrounding conditions are less favorable [19]. Thus, P-cad in similar manner like E-cad, could modulate its expression according to the degree of differentiation and surrounding conditions.

P-cad expression percentage did not appear to differ significantly in relation to histopathological grading. The finding resembles previous studies [8,10, 18]. And contradicts other [9]. Most of the grade-I cases were positive but had low expression percentage, while grade III cases showed P-cad under-expression, similar to Lo Muzio et al. findings [8,9]. Thus, P cadherin expression in oral SCC is unlike that in pancreatic and breast carcinomas. Over and aberrant P-cad expression in these tumors promotes the motility and aggressiveness of cancer cells [13, 20].

SCC in the oral cavity proper showed high cortactin overexpression percentage, unlike HNSCC [12, 21,22]. This discrepancy is due to biological site variation (pharynx and larynx), the difference in methodology (digital image analysis) [21,22] and scoring systems [12, 21].

This study indicates that OSCC had a considerable cytoskeletal reorganization and diffused cytoplasmic cortactin expression pattern. Thus, possibly more tumor aggressiveness as cortactin amplification was reported to promote carcinoma cell invasion [14,16]. As well as its amplification linked to poor prognosis [12,22 23]. In the agreement with Tsai et al. findings [24] in gastric carcinoma, all grade III OSCC showed cortactin positive expression. However, Yamada et al., [12] and Hofman et al., [21] reported lesser percentages in grade III OSCC (40% and 80% respectively). We did not find any differences in cortactin expression in relation to tumor grading.

Finally, this study can not show any correlation between P-cad and cortactin expressions. Thus in the initial phase of tumor growth the high expression of P-cad probably is a critical molecule in the formation of a tumor mass. Then, its cytoplasmic relocalization or loss of expression may be related to the later stages of tumor progression, which associate with cortactin over-expression to facilitate the growth invasion.

Conclusions:

Oral SCC had P-cad under expression and when cancerous cells expressed membranous P-cad (low expression) they had a focal heterogeneous distribution that changed with the degree of cell differentiation. Furthermore, OSCC had a great percentage of aberrant cortactin over-expression with a strong diffuse pattern that not related to tumor grading. The expressions of these adhesion molecules were unrelated to each other.

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References:

1. *Soames JV aand Southam JC, Oral Pathology: Oral epithelial tumors, melanocytic naevi, and malignant melanoma. 3rd ed, Oxford; Oxford Univ Press. 1998: 161.*
2. *Me´ndez E, Cheng C, Farwell DG, Ricks S, Agoff SN, Futran ND, Weymuller EA, Maronian NC, Zhao LP and Chen C, 2002. Transcriptional expression profiles of oral squamous cell carcinomas. Cancer. 2002; 95(7): 1482-94.*
3. *Humayun S and Prasad VR. Expression of p53 protein and Ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. Natl J Maxillofac Surg. 2011; 2(1):38-46.*
4. *Parades J. CDH3/P-cadherin overexpression in breast carcinomas: its regulatory mechanisms, the role in cell invasion, and the association with cancer stem cell properties. BMC Proc. 2010;4 (Suppl 2):017.*
5. *O'Donnell RK, Feldman M, Mick R and Muschel RJ. Immunohistochemical method identifies lymph vascular invasion in a majority of oral squamous cell carcinomas and discriminates between blood and lymphatic vessel invasion. J Histochem Cytochem. 2008; 56 (9): 803-10.*
6. *Choi S and Meyers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res. 2008; 87(1):14-32.*
7. *Thiery JP and Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006; 7(2):131-42.*
8. *Lo Muzio L, Campisi G, Farina A, Rubini C, Pannone G, Serpico R, Laino G, De Lillo A and Carinci F. P-cadherin expression and survival rate in oral squamous cell carcinoma: an immunohistochemical study. BMC Cancer. 2005; 21(5):63.*
9. *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. Histol Histopathol. 2004; 19(4):1089-99.*
10. *Munoz-Guerra MF, Marazuela EG, Fernández-Contreras ME and Gamallo C. P-cadherin expression reduced in squamous cell carcinoma of the oral cavity: an indicator of poor prognosis. Cancer. 2005; 103(5): 960-9.*
11. *Bauer R, Dowejko A, Driemel O, Boßerhoff AK and Reichert TE. Truncated P-cadherin is produced in oral squamous cell carcinoma. FEBS J. 2008; 275(16):4198-210.*
12. *Yamada SI, Yamamoto S, Kawasaki G, Mizuno A and Nemoto TK. Overexpression of cortactin increases invasion potential in oral squamous cell carcinoma. Pathol Oncol Res. 2010; 16(4):523-31.*
13. *Paredes J, Albergaria A, Oliveira JT, Jerónimo C, Milanezil F and Schmitt FC. P cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. Clin Cancer Res. 2005 Aug 15; 11(16):5869-77.*
14. *Greer RO Jr, Said S, Shroyer KR, Marileila VG and Weed SA. Overexpression of cyclin D1 and cortactin is primarily independent of gene amplification in salivary gland adenoid cystic carcinoma. Oral Oncol. 2007; 43(8):735-41.*
15. *Hideki K, Masahiro W and Akio T, Expression of P-cadherin and cyclin D1 in oral squamous cell carcinoma. J Osaka Odontological Society. 2002; 65:1-10.*
16. *Hirakawa H, Shibata K and Nakayama T. Localization of cortactin is associated with colorectal cancer development. Int J Oncol. 2009; 35(6):1271-6.*
17. *Williams HK, Sanders DS, Jankowski JA, Landini G and Brown AM. Expression of cadherins and catenins in oral epithelial dysplasia and squamous cell carcinoma. J Oral Pathol Med. 1998; 27(7):308-17.*
18. *Pyo SW, Hashimoto M, Kim YS, Kim CH, Lee SH, Johnson KR, Wheelock MJ 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 Craniomaxillofac Surg. 2007; 35(1):1-9.*
19. *Schmalhofer O, Brabletz S and Brabletz T. E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. Cancer Metastasis Rev. 2009; 28(1-2):151-66.*
20. *Taniuchi K, Nakagawa H, Hosokawa M, Nakamura T, Eguchi H, Ohigashi H, Ishikawa O, Katagiri T and Nakamura Y. Overexpressed P-cadherin/CDH3 promotes motility of pancreatic cancer cells by interacting with p120ctn and activating rho-family GTPases. Cancer Res. 2005; 65(8):3092-9.*
21. *Hofman P, Butori C, Havet K, Hofman V, Selva E, Guevara N, Santini J and Obberghen-Schilling EV. Prognostic significance of cortactin levels in head and neck squamous cell carcinoma: comparison with epidermal growth-factor receptor status. Br J Cancer. 2008; 98: 956–64.*
22. *Rothschild BL, Shim AH, Ammer AG, Kelley LC, Irby KB, Head JA, Chen L, Varella-Garcia M, Sacks PG, Frederick B, Raben D and Weed SA. Cortactin overexpression regulates actin-related protein 2/3 complex activities, motility, and invasion in carcinomas with chromosome 11q13 amplification. Cancer Res. 2006; 66: 8017–8025.*
23. *Rodrigo JP, García LA, Ramos S, Lazo PS and Suárez C. EMS1 gene amplification correlates with poor prognosis in squamous cell carcinomas of the head and neck. Clin Cancer Res. 2000; 6(8):3177-82.*
24. *Tsai WC, Jin JS, Chang WK, Chan DC, Yeh MK, Cherng SC, Lin LF, Sheu LF and Chao YC. Association of cortactin and fascin-1 expression in gastric adenocarcinoma: correlation with clinicopathological parameters. J Histochem Cytochem. 2007; 55(9): 955-62.*