Immunohistochemical Analysis of E-Cadherin Expression in Pleomorphic Adenoma and Mucoepidermoid Carcinoma of Salivary Glands

***[1]Saedeh Atarbashi Moghadam [2]Mahdi Pourmahdi [3]Mahdi Gholamian [3]Hootan Pedram [4]Fazeleh Atarbashi Moghadam**

*[1]Assistant Professor, Dept. of Oral & Maxillofacial Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: dr.atarbashi@gmail.com
[2]Assistant Professor, Dept. of Nutrition, School of Veterinary, Shahid Chamran University, Ahvaz, Iran.
[3]Undergraduate Student, School of Dentistry, Jondi Shapour University, Ahwaz, Iran.
[4]Assistant Professor, Dept. of Periodontics, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Abstract

Objective: E-cadherin is a classic cadherin that plays a key role in epithelial cell adhesion. This protein is being referred to as the suppressor of proliferation and invasion. Limited studies have investigated E-cadherin expression in salivary gland neoplasms. This study sought to assess the expression of E-cadherin and its possible role in progression and invasion of salivary gland neoplasms.

Methods: In this retrospective cross-sectional study, 15 samples of pleomorphic adenoma (PA) and 9 samples of mucoepidermoid carcinoma (MEC) were immunohistochemically stained for evaluation of E-cadherin expression. Degree of staining was calculated as the percentage of positively stained cell membranes out of a minimum of 1000 neoplastic cells.

Results: In normal salivary gland specimens, intense membrane staining was observed around the acinar mucous and serous cells as well as the ductal cells. Myoepithelial cells were negative. In PA, intense staining was noted along the membrane of attached cells forming the ducts, islands, cellular cords and cellular sheets but the stromal myoepithelial cells were negative. In MEC, epidermoid and intermediate cells showed intense membrane staining. Mucous cells also showed membrane staining. After statistical analysis, the percentage of positive cells was found to be 82.56±11.66 and 67.4±7.24 in MEC and PA, respectively. This difference was not statistically significant ($P>0.05$).

Conclusion: E-cadherin expression was not a suitable marker for differentiation of PA from MEC. It was only correlated with cell phenotype.

Key words: Cadherin, E-cadherin, Immunohistochemistry, Mucoepidermoid carcinoma, Pleomorphic adenoma, Salivary gland neoplasms

Please cite this article as follows:

Atarbashi Moghadam S, Pourmahdi M, Gholamian M, Pedram H, Atarbashi Moghadam F. Immunohistochemical Analysis of E-Cadherin Expression in Pleomorphic Adenoma and Mucoepidermoid Carcinoma of Salivary Glands. J Dent Sch 2013; 31(2):104-109.

Received: 25.12.2012 Final Revision: 25.02.2013 Accepted: 09.04.2013

Introduction:

Salivary gland tumors comprise an important group of oral neoplasms. Due to cellular and structural complexity, they show variable biological behavior. Various studies have been conducted in this respect (1) since information about the pathogenesis and biological behavior of tumors can help in accurate diagnosis and successful treatment (2). PA is the most common benign salivary gland tumor while MEC is the most common malignant salivary tumor (3). PA is comprised of epithelial and myoepithelial cells in a mesenchymal stroma (4). MEC contains epidermoid, intermediate and mucous cells showing a solid or cystic pattern (5). E-cadherin belongs to the cadherin super family. They are intra-membranous adhesion molecules that play important roles in cell adhesion, forming adherens junctions to bind cells within tissues

together. They are also involved in tissue development and cell morphology (6). Decreased expression of E-cadherin has been reported in oral squamous cell carcinoma, odontogenic tumors, soft tissue sarcomas, prostate cancer, breast cancer and colorectal cancer by several researchers (7-12). Decreased E-cadherin expression has been shown to be associated with an invasive behavior, high proliferation, poor differentiation, invasion, metastasis and poor prognosis (13).

The present study sought to assess the expression of E-cadherin marker in PA and MEC and its possible role in development, progression and invasion of these tumors.

Methods:

In this retrospective cross-sectional analytical study, 15 samples of PA and 9 samples of MEC were selected from the archives of the Pathology Department of Imam Khomeini and Jahad Daneshgahi hospitals in Ahvaz. All specimens were evaluated and confirmed by an oral pathologist. Specimens with inadequate sample size, poor quality or indefinite diagnosis were excluded from the study. The selected blocks were cut into 4 micron slices. For Immunohistochemical staining, E-cadherin marker and streptavidin biotinstandard method were used. Degree of staining of samples was then compared.

Immunohistochemistry (IHC)

The prepared slices were deparaffinized and dehydrated. For antigen stabilization, slides were immersed in buffered citrate solution with a pH of 6 and placed in a microwave for 10 min. After washing with phosphate buffered saline (PBS), the slides were incubated for an hour with E-cadherin monoclonal antibody (Dako Cytomation, Denmark) to evaluate the expression of E-cadherin. After washing with PBS for 5 min, slides were immersed in Zymed streptavidin and incubated for 10 min. In the next step, the slides were subjected to DAB (3, 3 diaminobenzidine hydrochloride) chromogenic reagent to produce a brown reaction product. After hematoxylin staining, dehydration with alcohol and clearing with Xylitol, samples were mounted on a slide. In order to ensure the staining technique, positive and negative controls were used at all phases. Oral mucosa specimens were considered as the positive control and slides without the primary antibody phase were considered as the negative control group (1).

Microscopic evaluation

Specimens were evaluated by two observers using Olympus CX21 light microscope (Tokyo, Japan). First, the stained areas were observed at 40× magnification. Tumoral cells that showed membrane staining (regardless of the intensity of staining) were considered as positive cells. Afterwards, at 400× magnification, 1000 neoplastic cells were counted in 10 random fields. Data were quantitatively analyzed and recorded as labeling index (LI) using the formula below:

$$LI = \frac{Number\ of\ positive\ tumoral\ cells\ regardless\ of\ the\ staining\ intensity}{1000\ tumoral\ cells}$$

Considering the high correlation coefficient between the two observers, the mean LI values were calculated, statistically analyzed and compared using SPSS version 16 software and t-test. $p<0.05$ was considered statistically significant.

Results:

The mean age of patients with PA and MEC was 34.6(3) and 47.78(7) yrs., respectively. The difference in this regard was not statistically significant ($p>0.05$). PA was more common among females while MEC was more prevalent among males. In terms of location, most cases of PA were in parotid gland while the majority of MECs were found in minor salivary glands. All understudy specimens including normal salivary gland tissue, PA and MEC tumors stained

positive for this marker but were different in terms of degree and pattern of staining. In evaluation of normal salivary gland tissue, intense membrane staining was noted in acinar mucous and serous cells as well as ductal cells. Myoepithelial cells were not stained (Figure 1).

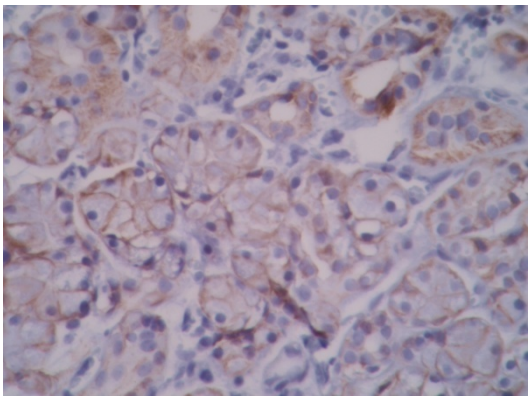

Figure 1- Membrane staining of acinar and ductal cells of normal salivary gland tissue showing E-cadherin expression (200× magnification)

In PA specimens, intense staining along the membrane of attached cells forming the ducts, islands, cellular cords and sheets was noted. But, the myoepithelial cells present in myxoid and chondroid stroma were not stained (Figure 2).

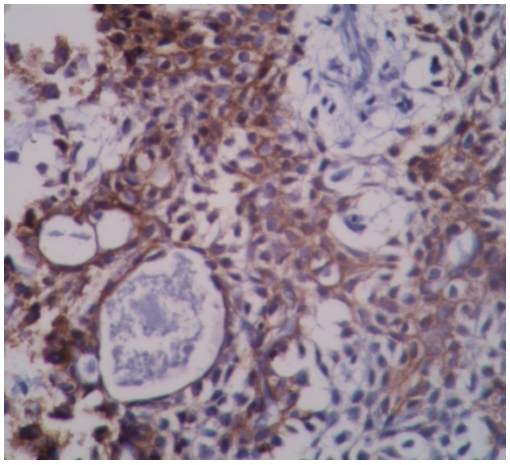

Figure 2- Membrane staining revealing the expression E-cadherin marker in PA (400× magnification)

In MEC, epidermoid and intermediate cells showed intense membrane staining. Occasional cytoplasmic or granular staining patterns were observed as well. Mucous cells also showed membrane staining (Figure 3). After the evaluation of slides and statistical analyses, percentage of positive cells in MEC and PA was found to be 82.56 (11.66) and 67.4 (7.24), respectively. The difference in this respect was not statistically significant (p>0.05). Degree (percentage) of staining of specimens was 15 to 100%.

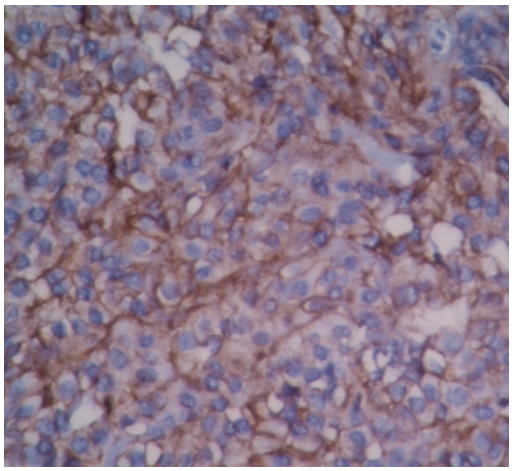

Figure 3- Membrane staining of epidermoid cells in MEC showing E-cadherin expression (200× magnification).

Discussion:

Tumors arising from salivary glands are not very common and include a series of neoplasms with different cell morphology and variable biological behavior (14). Among them, PA is the most common benign salivary tumor while MEC is the most common malignancy (3). E-cadherin is a 120 KD glycoprotein involved in epithelial cell adhesion. Decreased expression of this protein and related adhesion molecules in many neoplasms is associated with invasion, metastasis, low histological grade and poor prognosis (13). In normal epithelial tissues, this protein is expressed as a membrane protein (6).But, its abnormal expression as acytoplasmic or granular protein or its lack of expression have been seen in some human neoplasms as well (15). At present, only a few studies are available regarding the expression of this marker in

benign and malignant salivary gland tumors (5). In our study, E-cadherin expression was observed in both groups of benign and malignant tumors as well as normal salivary gland tissue. These results are in accordance with many previous studies (1, 5, 6, 16, 17). In all mentioned studies, E-cadherin expression was observed in normal salivary gland tissue in the form of membrane staining around the acinar mucous and serous as well as ductal cells. In a study by Andreadis et al. in 2006 (16) decreased or no expression of this marker was noted in the PA group mostly in cells with plasmacytoid and stromal differentiation. However, E-cadherin expression was intensely positive in other benign tumors such as Warthin's tumor. A mild to moderate decrease in E-cadherin expression was observed in MEC. Their obtained results regarding PA and MEC were similar to our findings. But an overall comparison between malignant tumors showed that decreased expression of this protein was associated with higher degree of invasion, cell phenotype and poor differentiation.

According to Shieh *et al*. in 2003 (5), abnormal expression of E-cadherin occurs in the majority of MEC cases. In another study, of 7 MEC cases, 6 showed normal and one showed abnormal expression of this marker (18). In a study by Furuse *et al*. in 2006 (17), membrane expression of this marker was reported in all benign and malignant salivary gland tumors. In our study, occasional cytoplasmic and granular expressions were found in some cases of MEC.

In some other studies like that of Economopoulou *et al*. in 2000 (6) the majority of salivary gland neoplasms regardless of their type, intensely expressed this marker and its decreased expression was seen in stromal and myoepithelial cells in PA. In MEC, no focal expression of marker was seen. Furthermore, increased or decreased E-cadherin expression was not associated with histological degree, differentiation or invasion in any of the malignant lesions. Thus, they concluded that in contrast to the malignant lesions in other parts of the body (such as colon adenocarcinoma), this protein is not a suitable marker for diagnosis or determination of prognosis of salivary tumors. The results of Prabhu *et al*. in 2009 were somehow different from the previous studies (13) because they reported lower expression of E-cadherin in malignant lesions compared to PA. They concluded that decreased expression of this protein occurs in differentiation of epithelial cells to stromal or myoepithelial phenotypes. Such conflicting results may be attributed to the microscopic pattern of PA and volume ratio of epithelial and myoepithelial cells since these tumors have very different microscopic patterns. In all evaluations, myoepithelial cells showed poor staining. Decreased expression of E-cadherin was also observed when evaluating its expression in other salivary gland tumors like Adenoid cystic carcinoma (ADCC) and comparing the primary tumors with recurrent and metastatic ones. Also, a significant association existed between gene methylation as well as decreased expression of E-cadherin and tumor progression and neural and vascular invasion. Furthermore, in some other investigations microscopic solid forms of this tumor showed decreased expression of this protein (19, 20).

Conclusion:

E-cadherin expression is not a suitable marker for the comparison of malignant MEC and benign PA. In contrast to many other malignant lesions, no significant correlation was found between malignancy of the tumor and decreased expression of this marker in salivary gland tumors. Also, this protein shows minimal stromal and myoepithelial staining and its decreased or increased expression depends on cell phenotype.

The present study had low reliability due to the

small number of samples. Future studies with a larger sample size are recommended on other salivary gland tumors and the relationship of E-cadherin expression with other adhesion molecules needs to be further investigated as well.

Conflict of Interest: "None Declared"

References:

1. Dultra FK, Barros AC, Schaer-Barbosa H, Figueiredo AL, Gurgel CA, Ramos EA, *et al*. Immunohistochemical assessment of CD1a-positive langerhans cells and their relationship with E-cadherin in minor salivary gland tumors. J Oral Pathol Med 2012; 41: 47-53.
2. Sengüven B, Oygür T. Investigation of interleukin-1 alpha and interleukin-6 expression and interleukin-1 alpha gene polymorphism in keratocystic odontogenic tumors and ameloblastomas. Med Oral Pathol Oral Cir Bucal 2011; 16: 467-472.
3. Morais Mde L, Azevedo PR, Carvalho CH, Medeiros L, Lajus T, Costa Ade L. Clinicopathological study of salivary gland tumors: an assessment of 303 patients. Cad Saude Publica 2011; 27:1035-1040.
4. Ito FA, Jorge J, Vargas PA, Lopes MA: Histopathological findings of pleomorphic adenomas of the salivary glands. Med Oral Pathol Oral Cir Bucal 2009; 14: 57-61.
5. Shieh YS, Chang LC, Chiu KC, Wu CW, Lee HC. Cadherin and catenin expression in mucoepidermoid carcinoma: correlation with histopathologic grade, clinical stage, and patient outcome. J Oral Pathol Med 2003; 32: 297-304.
6. Economopoulou P, Hanby A, Odell EW. Expression of E-cadherin, cellular differentiation and polarity in epithelial salivary neoplasms. Oral Oncol 2000; 36: 515-518.
7. Lopes FF, da Costa Miguel MC, Pereira AL, da Cruz MC, de Almeida Freitas R, Pinto LP, *et al*. Changes in immunoexpression of E-cadherin and beta-catenin in oral squamous cell carcinoma with and without nodal metastasis. Ann Diagn Pathol 2009; 13: 22-29.
8. Hakim SG, Kosmehl H, Sieg P, Trenkle T, Jacobsen HC, Attila Benedik G, *et al*. Altered expression of cell- cell adhesion molecules β-catenin/E-cadherin and related Wnt-signaling pathway in sporadic and syndromal keratocystic odontogenic tumors. Clin Oral Investig 2011; 15: 321-328.
9. Yoo J, Park S, Kang CS, Kang SJ, Kim BK. Expression of E-cadherin and p53 proteins in human soft tissue sarcomas. Arch Pathol Lab Med 2002; 126: 33-38.
10. Song Y, Oda Y, Hori M, Kuroiwa K, Ono M, Hosoi F *et al*. N-myc downstream regulated gene-1 / Cap43 may play an important role in malignant progression of prostate cancer, in its close association with E-cadherin. Hum Pathol 2010; 41: 214-222.
11. Facina G, Lopes-Costa PV, Dos Santos AR, De Vasconcelos-Valença RJ, Pinho-Sobral AL, Ferreira-Filho CP, *et al*. Immunohistochemical expression of E-cadherin in sclerosing adenosis, ductal carcinoma in situ and invasive ductal carcinoma of the breast. Diagn Cytopathol 2010; 38:235-238.
12. Gagliardi G, Kandemir O, Liu D, Guida M, Benvestito S, Ruers TG, *et al*. Changes in E-cadherin immunoreactivity in the adenoma- carcinoma sequence of the large bowel. Virchows Arch 1995; 426: 149-154.
13. Prabhu S, Kaveri H, Rekha K: Benign; malignant salivary gland tumors:comparison of immunohistochemical expression of e-cadherin. Oral Oncol 2009; 45: 594-599.

14. Patel RS, Rose B, Bawdon H, Hong A, Lee SC, Fredericks S, *et al*. Cyclin D1 and p16 expression in pleomorphic adenoma and carcinoma ex pleomorphic adenoma of parotid gland. Histopathology 2007; 51: 691-696.
15. Smith ME, Pignatelli M. The molecular histology of neoplasia: the role of the cadherin/catenin complex. Histopathology 1997; 31: 107-117.
16. Andreadis D, Epivatianos A, Mireas G, Nomikos A, Poulopous A, Yiotakis J. Immunohistochemical detection of E-cadherin in certain types of salivary gland tumors. J Laryngol Otol 2006; 120: 298-304.
17. Furuse C, Cury PR, Altemani A, dos Santos Pinto D Jr, de Araujo NS, de Araujo VC. Beta-catenin and E-cadherin expression in salivary gland tumors. Int J Surg Pathol 2006; 14: 212-217.
18. Tsubochi H, Suzuki T, Suzuki S, Ohashi Y, Ishibashi S, Moriya T, *et al*. Immunohistochemical study of basaloid squamous cell carcinoma, adenoid cystic and mucoepidermoid carcinoma in the upper aerodigestive tract. Anticancer Res 2000; 20: 1205-1211.
19. Zhang ZY, Wu YQ, Zhang WG, Tian Z, Cao J. The expression of E-cadherin-catenin complex in adenoid cystic carcinoma of salivary glands. Chin J Dent Res 2000; 3: 36-39.
20. Zhang CY, Mao L, Li L, Tian Z, Zhou XJ, Zhang ZY, *et al*. Promoter methylation as a common mechanism for inactivation E-cadherin in human salivary gland adenoid cystic carcinoma. Cancer 2007; 110: 87-95.