ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Expression of E-cadherin and matrix metalloproteinase-9 in oral squamous cell carcinoma and histologically negative surgical margins and association with clinicopathological parameters

NOOSHIN MOHTASHAM[1)], KAZEM ANVARI[2)], BAHRAM MEMAR[3)], NASROLLAH SAGHRAVANIAN[1)], NARGES GHAZI[4)], ALI BAGHERPOUR[5)], MAHSHID RAMTIN[6)]

[1)]*Department of Oral and Maxillofacial Pathology, Oral and Maxillofacial Diseases Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran*

[2)]*Department of Radiation Oncology, Radiation Oncology Research Center, Omid Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

[3)]*Department of Pathology, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

[4)]*Department of Oral and Maxillofacial Pathology, Dental Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran*

[5)]*Department of Oral and Maxillofacial Radiology, Dental Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran*

[6)]*Dentist, Private Office, Mashhad, Iran*

Abstract

Minimal residual cancer cells may not be detected in surgical margins of oral squamous cell carcinoma (OSCC) with routine histological examination. Using molecular markers at surgical margins can be helpful. We attempted to evaluate the MMP-9 and E-cadherin expression in OSCC samples and tumor-free surgical margins and association with clinicopathological factors. We examined E-cadherin and MMP-9 expression in 58 OSCCs including 19 grade I, 21 grade II and 18 grade III with histological tumor-free surgical margins by immunohistochemistry. Specimens were also divided in two groups: 19 samples as an early and 39 as an advanced stage. For E-cadherin in OSCCs and surgical margins, significant difference was observed between poor and moderate tumor differentiation. Different stages of OSCC demonstrated significant differences with higher expression in early stage tumors. For surgical margins, 82.1% of advanced and 84.2% of early stage samples demonstrated immunoreactivity. Both OSCC samples and surgical margins demonstrated significant differences for MMP-9 between stages with higher immunoreactivity in advanced stage, whereas there were not differences between different grades in surgical margins. E-cadherin and MMP-9 expression at histologically negative surgical margins shows the significance of these markers for prognostic values in OSCC patients with E-cadherin being the preferred predictor.

Keywords: oral squamous cell carcinoma, matrix metalloproteinase-9, E-cadherin.

Introduction

Squamous cell carcinoma (SCC) is the sixth most frequent malignant tumor of the head and neck region. The rate of the mortality and five-year survival has not been improved markedly over the last few decades [1–3].

Head and neck squamous cell cancers (HNSCC) have a high rate of local recurrence due to inadequate surgical resection [4].

Histological assessment of surgical margins leads to complete tumor excision, but minimal residual cancer cells may not be detected with routine histological examination [5]. Recurrences have been demonstrated at the primary site in histologically diagnosed tumor-free margins [6]. Using molecular markers for identification of malignant cells plays an important role in performing therapeutic options and reduces the incidence of cancer recurrences [5].

The invasion and spread of tumor cells require degradation and breakdown of the extracellular matrix (ECM) [7]. Multiple classes of ECM-degrading enzymes including matrix metalloproteinases (MMPs) are involved in migration of tumor cells [8].

E-cadherin glycoprotein is a Ca^{2+}-dependent intercellular adhesion molecule in epithelial cells. It is involved in the regulation of epithelial cell-to-cell adhesion. There is a relationship between this marker and invasion of tumoral cells. Reduction the expression of E-cadherin has been shown in many malignant tumors [9, 10].

The aim of this study was to evaluate the expression of mesenchymal marker MMP-9 and epithelial marker E-cadherin in oral squamous cell carcinoma (OSCC) samples and their surgical margins with no histological evidence of malignancy and association with grade and stage of tumor. Although not the main aim of our study, the correlation between these markers and age, gender of patients, site of tumor were investigated.

Materials and Methods

The files of Department of Pathology, Omid Hospital, Mashhad University of Medical Sciences, Iran were

searched for cases of oral squamous cell carcinoma (OSCC) that were operated for their tumor during 2000 to 2012.

The inclusion criteria of patients were based on primary disease, tumor site (oral cavity), histology of the primary tumor (squamous cell carcinoma), and histological status of surgical resection margins. All patients included in this study had a final pathology report of histologically normal resection margins, meaning none of the patients had a positive margin. Patients diagnosed with histological variants of squamous cell carcinoma (SCC) or recurrent tumors were excluded.

In our hospital for OSCC patients, the *UK Royal College of Pathologists* issued guideline for head and neck carcinoma in 1998 was used to define the margin status, including a histological distance from invasive carcinoma to surgical margins of more than 5 mm as clear [11]. Also for all patients, at the time of surgery, three to four margin samples (M1–M4) were taken from the surgical defect after tumor excision.

Fifty-eight OSCC samples including 19 grade I, 21 grade II, 18 grade III with histological tumor-free tissue (5 mm) at their surgical margins were retrieved and histologically evaluated by two experienced professionals.

Specimens were divided in two groups: stage I and stage II were considered as early stages and stage III, IVA and IVB as advanced stages [12]. Clinicopathological information including gender, age, and tumor location was gathered.

For IHC, four slices with a thickness of 4 μm were prepared from each paraffin-embedded section. The sections were fixed on poly-L-Lysine-coated glass slides. Deparaffinized and rehydrated slides were incubated for 30 minutes in 3% hydrogen peroxide/methanol to stop endogenous peroxidase activity and then irrigated with phosphate-buffered saline (PBS) for 20 minutes. For antigen retrieval, the sections were microwaved in citrate solution (0.01 M, pH 6.0) for 35 minutes. Specimens were incubated with the primary antibodies for one hour at room temperature. The sections were rinsed three times with PBS at room temperature. The secondary antibody was applied, and immune complexes were identified by Streptavidin peroxidase (Novo Link Polymer detection system). After three times washing with PBS, the immune reactivity was visualized by 3,3'-Diamino-benzidine and hydrogen peroxide. Finally, slides were counterstained with Hematoxylin and cover slipped with a synthetic mounting media. The immunohistochemical staining kit was the Novo Link Polymer detection system (RE7140-K 250T), which is an updated version of Biotin Labeled Streptavidin (LSAB). MMP-9 antibody (Novocastra Laboratories Ltd., 15W2, dilution 1:20) and E-cadherin antibody (NCL-E-Cad, Novocastra Laboratories Ltd.; dilution 1:50) were used according to the manufacturer's instruction (Novocastra).

Each stained slide was evaluated by at least two independent investigators. For each marker expression, quantification of the number of positive cells was counted under light microscopy in five microscopic fields with a magnification of 400×.

MMP-9-stained slides were evaluated according to cytoplasmic positivity of epithelial cells. The positive cells were graded as 0: no positive staining; 1: <10%; 2: 11–50%; 3: 51–80%; 4: >80% [13].

E-cadherin stained slides were investigated according to cell membrane positivity (preserved), cell membrane and nuclear positivity (altered) [14].

Intensity of E-cadherin expression was quantified using Intensity Reactivity Score (IRS), where staining intensity (SI) was assessed to be negative (=0), weak (=1), moderate (=2) or strong (=3) and reactivity was determined by percentage of positive cells (PP). IRS was calculated by multiplying SI with PP [15, 16].

The staining pattern was evaluated by comparing the cellular localization of immunostaining with those of normal tissue as an external positive control.

All analyses were performed using data processing program SPSS/PC version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Wilcoxon, Mann–Whitney, Kruskal–Wallis, Spearman's rank correlation coefficient test and Fisher's exact tests were used to assess the statistical significant differences between E-cadherin and MMP-9 expression and clinicopathological parameters in the studied groups, with $p<0.05$ being considered statistically significant.

Results

The association of E-cadherin expression with tumor stage and differentiation grade

Evaluation of IRS for E-cadherin expression in OSCC sections and surgical margins by Wilcoxon test revealed statistically significant difference between poor ($p=0.01$) and moderate ($p=0.02$) tumor differentiation, while well differentiated OSCC (grade I) did not show significant difference ($p>0.05$) (Table 1).

Table 1 – ***The association of MMP-9 and E-cadherin expression with differentiation grade in OSCC sections and surgical margins***

P-value	*n*	Studied groups	
0.041	19	I	Tumor (MMP-9)
	21	II	
	18	III	
0.357	19	I	Surgical margin (MMP-9)
	21	II	
	18	III	
0.083	19	I	Tumor and surgical margin (E-cadherin)
0.011	21	II	
0.026	18	III	

Altered E-cadherin expression was detected mainly in different grades of OSCC samples (Figure 1). In surgical margins of OSCC grade II and III altered immuno-expression was observed, whereas surgical margins of OSCC grade I retained preserved reactivity (Figure 2). Statistically significant difference was detected between site of E-cadherin expression and different grades in OSCC samples ($p=0.01$) and surgical margins ($p=0.02$).

According to Mann–Whitney test, different stages of OSCC (advanced and early stages) demonstrated significant difference ($p<0.000$) with higher E-cadherin expression in early stage tumors.

In OSCC specimens, the majority of the advanced stage cases (92.3%) showed altered E-cadherin expression while in early stage tumors both altered and preserved reactivity were detected equally.

For surgical margins, 82.1% of advanced stage and 84.2% of early stage samples demonstrated altered and preserved immunoexpression respectively. Fisher's exact test revealed a significant difference between stage of tumor and location of E-cadherin expression both in surgical margins (p=0.000) and OSCC samples (p=0.000) (Table 2).

Table 2 – ***Correlation between E-cadherin and MMP-9 expression and tumor stage in OSCC sections and surgical margins***

P-value	Mean rank	*n*	Studied groups	
0.000	18.43	19	Early	Tumor (E-cadherin)
	83.22	39	Advanced	
0.000	89.42	19	Early	Surgical margin (E-cadherin)
	97.22	39	Advanced	
0.001	79.18	19	Early	Tumor (MMP-9)
	72.34	39	Advanced	
0.001	00.19	19	Early	Surgical margin
	62.34	39	Advanced	

The association of MMP-9 expression with tumor stage and differentiation grade

According to Kruskal–Wallis test, OSCC grade I showed a significant difference with grade II and III (p=0.04), whereas there was not any significant difference between different grades in surgical margins of OSCC (p>0.05) (Table 1).

Mann–Whitney test showed that both OSCC samples and their surgical margins demonstrated a significant difference between early and advanced stages (p=0.001), with higher immunoreactivity in advanced stage (Table 2, Figures 3 and 4).

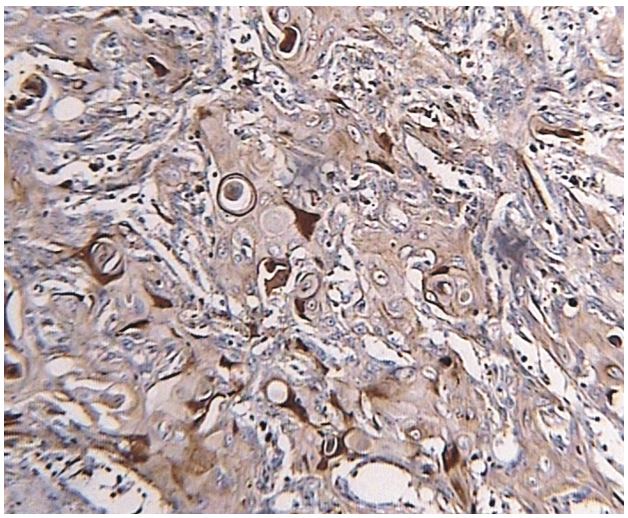

Figure 1 – ***E-cadherin expression in OSCC grade II and advanced stage (100×).***

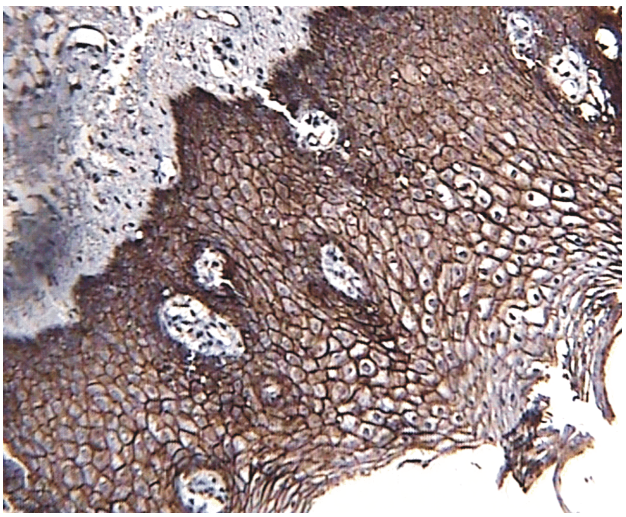

Figure 2 – ***E-cadherin expression in surgical margin of OSCC grade III and advanced stage (100×).***

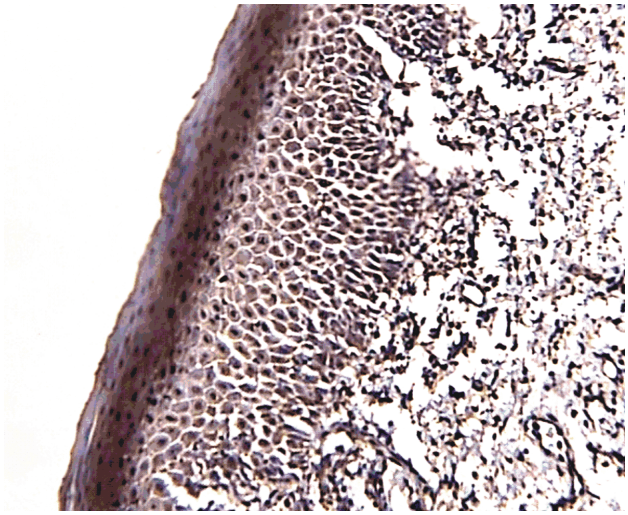

Figure 3 – ***MMP-9 expression in surgical margin of OSCC grade III and advanced stage (100×).***

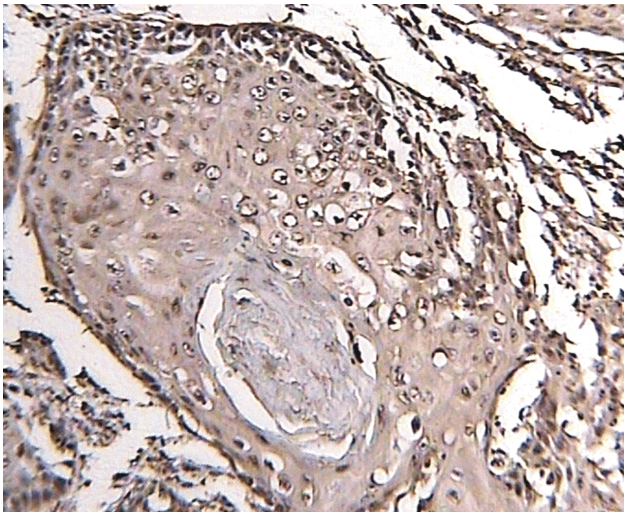

Figure 4 – ***MMP-9 expression in OSCC grade II and advanced stage (100×).***

Correlation between MMP-9 and E-cadherin

Spearman's rank correlation coefficient test did not show a significant correlation between MMP-9 and E-cadherin expression for different grades and stages of OSCC samples and also for surgical margins (p>0.05).

Correlation between MMP-9, E-cadherin expression and other clinicopathological parameters

OSCC cases comprised of 34 men and 24 women. The mean age for patients was 61 years (range 32–77 years). Two groups were considered for primary location of

tumors: 1 – tongue (28); 2 – other mouth areas (30). The expression of MMP-9 and E-cadherin was positively correlated with primary site of tumor when analyzed by the Spearman's rank correlation coefficient test. The greatest Pearson's correlation coefficient (r) was between E-cadherin expression in OSCC samples and surgical margin in tongue specimens (r=81). No relationship was observed between MMP-9, E-cadherin expression and other parameters including patient gender and age (p>0.05).

Discussion

Squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm of oral cavity [15]. Epithelial carcinogenesis is a multistep process. Progression from normal oral epithelium to cancer is believed to result from cumulative genetic alterations [17–19].

Prognostic markers that could act as a therapeutic target could play an important role in effective treatment strategies of oral cancer [20]. The prediction of oral squamous cell carcinoma (OSCC) behavior is different using conventional clinical and histopathological parameters [21]. Therefore, studies on molecular biomarkers including cell adhesion and matrix degradation markers have performed as potential tools to predict the prognosis of patients with OSCC. Immunohistochemistry (IHC) as an available tool can provide useful information about prognostic tumor markers associated with clinical outcome of OSCC [21].

Despite advances in therapeutic options for head and neck squamous cell carcinoma over the last decades, the five-year survival rate of patients has been improved moderately. This is in part due to a high percentage (10–50%) of loco-regional and distant recurrences after surgery even in patients with histopathologically negative surgical margins [6, 22].

Various studies showed the limitation of standard histopathology for evaluation of surgical margins [23].

To detection minimal residual tumor cells at histo-pathological normal surgical margins, molecular-based technologies have been developed [24]. It has been demonstrated that molecular investigation of apparently tumor-free margins of excised tissue plays an important role in prediction of local tumor recurrence in head and neck squamous cell carcinoma patients [25, 26]. It has been reported that the grading of epithelial dysplasia in the surgical margins may have value in assessing the risk for developing local recurrences [27].

Most of prior studies investigated head and neck squamous cell carcinomas *versus* general oral SCCs. Therefore, differences in the primary cancers may cause discrepancy. Therefore, in the current study only OSCC samples were evaluated. Moreover, we attempted to evaluate expression of MMP-9 as a mesenchymal marker and E-cadherin as an epithelial marker in OSCC samples and histologically negative surgical margins to show the significance of both epithelial and mesenchymal markers for prognostic values in these patients.

Miyamoto *et al.* [28] suggested that molecular imaging has high accuracy for complete tumor resection and improvement of head and neck cancer. They also recommended that the complexity of these new molecular approaches limit their implementation in routine clinical use.

In our study, we applied immunohistochemistry to identify residual tumor in surgical margins. Compared to histology as the gold standard this technique showed more accurate results.

As previously mentioned, E-cadherin plays an important role in intercellular adhesion in epithelial tissues. Dysfunction and reduced immunoexpression of E-cadherin is involved in malignancy and carcinogenesis.

In the current study, reduction of E-cadherin expression was significantly associated with higher stage and grade of disease. Several studies also demonstrated that reduced immunoexpression of this marker in oral squamous cell carcinoma correlates with higher stage and grade of disease, lymph node metastasis, tumor invasive behavior and carcinogenesis, which are in consistent with our study [15, 16, 29, 30].

On the other hand, Ukpo *et al.* [31] suggested that E-cadherin expression may not be a predictor for nodal or distant metastasis in OSCCs.

The role of various types of MMPs is important for controlled matrix remodeling. Gelatinases MMP-2 and MMP-9 are the essential parts of basement membranes. These are the only MMPs that can degrading native collagen type IV and play an important role in cell migration and invasion [32].

Here, we showed the overexpression of MMP-9 in advanced stage tumors, which is in consistent with previous studies [7, 25, 33].

Conclusions

Our findings suggest that E-cadherin and MMP-9 expression at histological-negative surgical margins of OSCC can detect the presence and absence of minimal residual cells in surgical margins, diagnosed as tumor-free by conventional histopathological evaluation. Therefore, they may become a helpful biomarker to identify subjects at risk of new neoplastic evolution, with E-cadherin being the preferred predictor.

Acknowledgments

The research results given in this paper were obtained from doctoral thesis by a Grant (No. 900088) supported from the Vice Chancellor of Mashhad University of Medical Sciences, Iran.

References

[1] Tanaka T, Ishigamori R, *Understanding carcinogenesis for fighting oral cancer*, J Oncol, 2011, 2011:603740.

[2] Boslooper K, King-Yin Lam A, Gao J, Weinstein S, Johnson N, *The clinicopathological roles of alpha-B-crystallin and p53 expression in patients with head and neck squamous cell carcinoma*, Pathology, 2008, 40(5):500–504.

[3] Chu PY, Hsu NC, Tai HC, Yeh CM, Lin SH, Hou MF, Yeh KT, *High nuclear protein kinase Cθ expression may correlate with disease recurrence and poor survival in oral squamous cell carcinoma*, Hum Pathol, 2012, 43(2):276–281.

[4] Nathan CO, Liu L, Li BD, Abreo FW, Nandy I, De Benedetti A, *Detection of the proto-oncogene eIF4E in surgical margins may predict recurrence in head and neck cancer*, Oncogene, 1997, 15(5):579–584.

[5] Tan HK, Saulnier P, Auperin A, Lacroix L, Casiraghi O, Janot F, Fouret P, Temam S, *Quantitative methylation analyses of resection margins predict local recurrences and disease-specific deaths in patients with head and neck squamous cell carcinomas*, Br J Cancer, 2008, 99(2):357–563.

[6] Slootweg PJ, Hordijk GJ, Schade Y, van Es RJ, Koole R, *Treatment failure and margin status in head and neck cancer. A critical view on the potential value of molecular pathology*, Oral Oncol, 2002, 38(5):500–503.
[7] Gu ZD, Chen KN, Li M, Gu J, Li JY, *Clinical significance of matrix metalloproteinase-9 expression in esophageal squamous cell carcinoma*, World J Gasteroenterol, 2005, 11(6):871–874.
[8] Birkedal-Hansen H, *Proteolytic remodeling of extracellular matrix*, Curr Opin Cell Biol, 1995, 7(5):728–735.
[9] Sun GY, Wu JX, Wu JS, Pan YT, Jin R, *Caveolin-1, E-cadherin and β-catenin in gastric carcinoma, precancerous tissues and chronic non-atrophic gastritis*, Clin J Cancer Res, 2012, 24(1):23–28.
[10] Huang MF, Zhu YQ, Chen ZF, Xiao J, Haung X, Xiong YY, Yang GF, *Syndecan-1 and E-cadherin expression in differentiated type of early gastric cancer*, World J Gasteroenterol, 2005, 11(19):2975–2980.
[11] Helliwell TR, Woolgar JA, *Standards and datasets for reporting cancers. Datasets for histopathology reports on head and neck carcinomas and salivary neoplasms*, 2nd edition, Royal College of Pathologists, London, 2005.
[12] Mendenhall WM, Werning JW, Pfister DG, Treatment of head and neck cancer. In: DeVita VT, Lawrence TS, Rosenberg SA (eds), *DeVita, Hellman, and Rosenberg's cancer: principles and practice of oncology*, 8th edition, Lippincott Williams & Wilkins, Philadelphia, 2008, 809–825.
[13] Yamamoto H, Vinitketkumnuen A, Adachi Y, Taniguchi H, Hirata T, Miyamoto N, Nosho K, Imsumran A, Fujita M, Hosokawa M, Hinoda Y, Imai K, *Association of matrilysin-2 (MMP-26) expression with tumor progression and activation of MMP-9 in esophageal squamous cell carcinoma*, Carcinogenesis, 2004, 25(12):2353–2360.
[14] Tanaka N, Odajima T, Ogi K, Ikeda T, Satoh M, *Expression of E-cadherin, alpha-catenin, and beta-catenin in the process of lymph node metastasis in oral squamous cell carcinoma*, Br J Cancer, 2003, 89(3):557–563.
[15] Wang C, Liu X, Chen Z, Huang H, Jin Y, Kolokythas A, Wang A, Dai Y, Wong DT, Zhou X, *Polycomb group protein EZH2-mediated E-cadherin repression promotes metastasis of oral tongue squamous cell carcinoma*, Mol Carcinog, 2013, 52(3):229–236.
[16] Huber GF, Züllig L, Soltermann A, Roessle M, Graf N, Haerle SK, Studer G, Jochum W, Moch H, Stoeckli SJ, *Down regulation of E-cadherin (ECAD) – a predictor for occult metastatic disease in sentinel node biopsy of early squamous cell carcinomas of the oral cavity and oropharynx*, BMC Cancer, 2011, 11:217–218.
[17] Mohtasham N, Mahdavi-Shahri N, Salehinejad J, Ejtehadi H, Torabi-Parizi M, Ghazi N, *Detection of nucleoproteins in squamous cell carcinoma, and dysplastic and normal mucosa in the oral cavity by methyl green-pyronin staining*, J Oral Sci, 2010, 52(2):239–243.
[18] Ambrosch P, Schlott T, Hilmes D, Ruschenburg I, *p16 alterations and retinoblastoma protein expression in squamous cell carcinoma and neighboring dysplasia from the upper aerodigestive tract*, Virchows Arch, 2001, 438(4):343–349.
[19] Abrahao AC, Bonelli BV, Nunes FD, Dias EP, Cabral MG, *Immunohistochemical expression of p53, p16 and hTERT in oral squamous cell carcinoma and potentially malignant disorders*, Braz Oral Res, 2011, 25(1):34–41.
[20] Chen CJ, Hsu LS, Lin SH, Chen MK, Wang HK, Hsu JD, Lee H, Yeh KT, *Loss of nuclear expression of Krüppel-like factor 4 is associated with poor prognosis in patients with oral cancer*, Hum Pathol, 2012, 43(7):1119–1125.
[21] Olivera L.R, Riberio-Silva A, *Prognostic significance of immunohistochemical biomarkers in oral squamous cell carcinoma*, Int J Oral Maxillofac Surg, 2011, 40(3):298–307.
[22] Hinerman RW, Mendenhall WM, Morris CG, Amdur RJ, Werning JW, Villaret DB, *Postoperative irradiation for squamous cell carcinoma of the oral cavity: 35-year experience*, Head Neck, 2004, 26(11):984–994.
[23] Bradley PJ, MacLennan K, Brakenhoff RH, Leemans CR, *Status of primary tumour surgical margins in squamous head and neck cancer: prognostic implications*, Curr Opin Otolaryngol Head Neck Surg, 2007, 15(2):74–81.
[24] Rodrigo JP, Ferlito A, Suárez C, Shaha AR, Silver CE, Devaney KO, Bradley PJ, Bocker JM, McLaren KM, Grénman R, Rinaldo A, *New molecular diagnostic methods in head and neck cancer*, Head Neck, 2005, 27(11):995–1003.
[25] 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(8):1727–1730.
[26] Nathan CO, Amirghahri N, Rice C, Abreo FW, Shi R, Stucker FJ, *Molecular analysis of surgical margins in head and neck squamous cell carcinoma patients*, Laryngoscope, 2002, 112(12):2129–2140.
[27] Braakhuis BJ, Bloemena E, Leemans CR, Brakenhoff RH, *Molecular analysis of surgical margins in head and neck cancer: more than a marginal issue*, Oral Oncol, 2010, 46(7):485–491.
[28] Miyamoto S, Sperry S, Yamashita T, Reddy NP, O'Malley BW Jr, Li D, *Molecular imaging assisted surgery improves survival in a murine head and neck cancer model*, Int J Cancer, 2012, 131(5):1235–1242.
[29] Vered M, Allon I, Buchner A, Dayan D, *E-cadherin in oral SCC: an analysis of the confusing literature and new insights related to its immunohistochemical expression*, Histol Histopathol, 2012, 27(2):141–150.
[30] Sakamoto K, Imanishi Y, Tomita T, Shimoda M, Kameyama K, Shibata K, Sakai N, Ozawa H, Shigetomi S, Fujii R, Fujii M, Ogawa K, *Overexpression of SIP1 and downregulation of E-cadherin predict delayed neck metastasis in stage I/II oral tongue squamous cell carcinoma after partial glossectomy*, Ann Surg Oncol, 2012, 19(2):612–619.
[31] Ukpo OC, Thorstad WL, Zhang Q, Lewis JS Jr, *Lack of association of cadherin expression and histopathologic type, metastasis, or patient outcome in oropharyngeal squamous cell carcinoma: a tissue microarray study*, Head Neck Pathol, 2012, 6(1):38–47.
[32] Genersch E, Hayess K, Neuenfeld Y, Haller H, *Sustained ERK phosphorylation is necessary but not sufficient for MMP-9 regulation in endothelial cells: involvement of Ras-dependent and -independent pathways*, J Cell Sci, 2000, 113(Pt 23):4319–4330.
[33] Ogbureke KU, Weinberger PM, Looney SW, Li L, Fisher LW, *Expressions of matrix metalloproteinase-9 (MMP-9), dentin sialophosphoprotein (DSPP), and osteopontin (OPN) at histologically negative surgical margins may predict recurrence of oral squamous cell carcinoma*, Oncotarget, 2012, 3(3):286–298.

Corresponding author
Narges Ghazi, Assistant Professor, Department of Oral and Maxillofacial Pathology, Dental Research Center, School of Dentistry, Mashhad University of Medical Sciences, Vakilalbad Avenue, P.O. Box 911735–984, Mashhad, Iran; Phone +98 511 8829501, Fax +98 511 8829500, e-mail: nargess_ghazi@yahoo.com, ghazin@mums.ac.ir

Received: May 16, 2013 *Accepted: January 27, 2014*