THE STUDY OF E-CADHERIN EXPRESSION IN GLOTTIC LARYNGEAL SQUAMOUS CELL CARCINOMA

Elvir Zvrko[1], Anton Mikic[2], Ljiljana Vuckovic[3], Milan Knezevic[4], Vojko Djukic[2]
[1]Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical center of Montenegro, Podgorica, Montenegro,
[2]Institute for Otorhinolaryngology and Maxillofacial Surgery, School of Medicine,
University of Belgrade, Belgrade, Serbia [3]Center for Pathology and Forensic Medicine, Clinical center of Montenegro, Podgorica, Montenegro [4]School of Medicine, University of Kragujevac, Kragujevac, Serbia

EKSPRESIJA E-KADHERINA KOD SKVAMOCELULARNIH KARCINOMA GLOTIČNE REGIJE LARINKSA

Elvir Zvrko[1], Anton Mikić[2], Ljiljana Vučković[3], Milan Knežević[4], Vojko Đukić[2]
[1] Klinika za otorinolaringologiju i maksilofacijalnu hirurgiju, Klinički Centar Crne Gore, Podgorica, Crna Gora
[2] Institut za Otorinolaringologiju i Maksilofacijalnu hirurgiju, Medicinski fakultet, Univerzitet u Beogradu, Beograd, Srbija
[3] Centar za Patologiju i forenzičku medicinu, Klinički Centar Crne Gore, Podgorica, Crna Gora,
4 Medicinski fakultet, Univerzitet u Kragujevcu, Kragujevac, Srbija

Received / Primljen: 16. 04. 2009. Accepted / Prihvaćen: 22. 07. 2009.

ABSTRACT

Background: E-cadherin is a 120 kDa transmembrane protein that is thought to play an important role in malignant progression of tumours and in tumour differentiation. A reduced or absent expression of E-cadherin has been observed in several carcinomas, including squamous cell carcinoma of the head and neck.

Objective: The aim of this study was to analyse the clinicopathologic significance of E-cadherin expression in squamous cell carcinomas with a primary location in the glottic region of the larynx.

Materials and methods: E-cadherin expression was determined by immunohistochemistry in paraffin-embedded tissue specimens from 40 patients with squamous cell carcinoma of the glottic larynx. A staining score was given based on the percentage of cells stained (0–100%). All stained cells were considered positive regardless of the intensity of the staining. Using the mean expression of E-cadherin as a cut-off, 17 (42.5%) tumours were classified into the "high E-cadherin" group and 23 (57.5%) into the "low E-cadherin" group.

Results: E-cadherin expression varied greatly among the tissue samples, with scores ranging from 2 to 72 (median 23). The mean expression score for E-cadherin was 27.35 (standard deviation [SD]=20.15). Decreased E-cadherin expression was significantly correlated with more aggressive tumours, including tumours staged as T3 or T4 (p = 0.038) and those with advanced clinical stage (TNM stage III and IV) (p = 0.010). The results of a stepwise logistic regression analysis showed that only the presence of lymph node metastasis was an independent predictor for tumour recurrence (p=0.019). A Cox proportional hazards model confirmed that the presence of cervical lymph node metastases (P=0.003) and age ≤ 59 years (P=0.006) were statistically significant independent predictors of a reduced disease-specific survival.

Conclusion: Expression of E-cadherin may be useful to identify patients with aggressive disease, allowing more effective treatment strategies to be implemented.

SAŽETAK

"Ekspresija E-kadherina kod skvamocelularnih karcinoma glotične regije larinksa".

Uvod: E-kadherin je transmembranski protein molekularne mase 120 kDa koji ima važnu ulogu u progresiji i diferencijaciji tumora. Odsustvo ili smanjena ekspresija E-kadherina nađena je za veliki broj neoplazmi uključujući i karcinome glave i vrata.

Cilj istraživanja bio je da se analizira kliničko-patološki značaj ekspresije E- kadherina u pacijenata sa planocelularnim karcinomom grkljana lokalizovanim u glotisu.

Materijal i metode: Ekspresija E- kadherina analizirana je imunohistohemijski u 40 pacijenata sa glotisnim karcinomom grkljana. Rezultat imunohistohemijske ekspresije E-kadherina pretstavljao je procenat obojenih ćelija (0–100%). Sve obojene ćelije su uključene u brojanje bez obzira na intenzitet. U odnosu na srednju vrijednost ekspresije ispitanici su podijeljeni u dvije grupe. U 17 (42.5%) slučajeva se radilo o visokoj ekspresiji (procenat obojenih ćelija veći od srednje vrijednosti) a 23 (57.5%) pacijenta su imali nisku ekspresiju E- kadherina (procenat manji od srednje vrijednosti).

Rezultati: Ekspresija E- kadherina u posmatranom materijalu varirala je 2 do 72 (mediana 23). Srednja vrijednost ekspresije E- kadherina iznosila je 27.35 (standardna devijacija [SD]= 20.15). Značajno slabija ekspresija E-kadherina nađena je u pacijenata sa lokalno proširenim tumorom (kategorija T3 i T4) (χ2-test p= 0.038) kao i u pacijenata sa uznapredovalom bolešću (TNM stadijum III i IV) (χ2-test p= 0.010). Multivarijantnom logističkom regresionom analizom dobili smo da je prisustvo metastaza na vratu jedini nezavisni prediktor relapsa bolesti (p=0.019). Rezultati Cox-ove regresione analize pokazuju da su nezavisni prediktori kraćeg preživljavanja bez bolesti prisustvo metastaza na vratu (P=0.003) i starosna dob ≤ 59 godina (P=0.006).

Zaključak: Određivanje ekspresije E- kadherina moglo bi da pomogne u otkrivanju pacijenata sa agresivnim tipom bolesti što bi vodilo određivanju optimalnog

UDK 577.112.4:616.22-006.6 / Ser J Exp Clin Res 2009; 10 (3): 89-94

Correspondence: Dr Elvir Zvrko,Ljubljanska bb, Clinical Center of Montenegro, 81000 Podgorica, Montenegro,
e-mail: elvirz@cg.yu, phone: +382 69 401 431

Larger studies are required to confirm the role of E-cadherin expression in predicting the behaviour of laryngeal squamous cell carcinomas.

Key words: *Laryngeal carcinomas, Cell adhesion molecule, E-cadherin, Immunohistochemistry, Head and neck.*

načina liječenja. Uloga E-kadherina u procjeni biološkog ponašanja karcinoma larinksa treba da bude potvrđena dodatnim istraživanjima.

Ključne reči: *karcinom larinksa, ćelijski adhezioni molekul, E-kadherin, imunohistohemija, glava i vrat*

INTRODUCTION

The development of cancer involves multiple coordinated cellular processes. Identification of the molecular mechanisms involved in laryngeal cancer progression will contribute to a better understanding of its biological behaviour. Multiple steps are required to induce tumour invasion and metastasis, including the expression of a variety of gene products that include adhesion molecules. Weakening of cell-cell and cell-extracellular matrix adhesions is obviously imperative for tumour cells to metastasise. Several families of cell adhesion molecules have been described. These include cadherins, integrins, adhesion molecules belonging to the immunoglobulin superfamily, selectins, and CD44. The cadherins are a group of calcium-dependent adhesion molecules that mediate homotypic cell-cell interactions, although heterotypic binding between different cadherin molecules is possible. They have an extracellular domain (N-terminal) that is implicated in homophilic binding and a cytoplasmic tail (C-terminal) that interacts with cytoskeletal proteins via intracellular proteins termed catenins (α, β, γ), which form the E-cadherin-catenin complex (1). There are four cadherin subclasses (classical cadherins, classical-related cadherins, desmosomal cadherins and modified cadherins). Among these adhesion molecules, epithelial cadherin (E-cadherin) is the most important, since it is expressed in all adult human epithelial tissues. A reduced or absent expression, or an abnormal location, of the E-cadherin/catenin complex has been observed in several carcinomas, including malignant tumours of the female genital tract (2), stomach (3), nasopharynx (4), bladder (5), prostate (6), lung (7), colon (8), breast (9) and squamous cell carcinomas of the head and neck (10-12).

In the present study, we used immunohistochemistry to examine the expression of E-cadherin in invasive glottic laryngeal squamous cell carcinomas, and we correlated our results with clinicopathological parameters.

MATERIALS AND METHODS

Patients

Forty patients with squamous cell carcinoma of the glottic larynx were selected from the pathological files of the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Center of Montenegro in Podgorica. All selected patients underwent complete resection as primary treatment in the period from 2001 to 2008. All patients had a single primary tumour, none had undergone treatment prior to surgery, and all had microscopically clear surgical margins. None of the patients was thought to have had distant metastases at the time of surgery. The clinical information, including sex, age, histologic grade, primary tumour (T) classification, nodal (N) status, TNM stage, and oncological outcome were obtained retrospectively from clinical records. Pathological staging was determined according to the 6th TNM Classification of Malignant Tumours of the International Union Against Cancer. Twenty patients had early cancer (Stage I or II) and 20 had advanced cancer (Stage III or IV). Treatment decision-making was based on clinical stage and on the presence or absence of lymph node metastases at the time of diagnosis. Partial laryngectomy was performed in 26 patients, and total laryngectomy in 14 patients. Nine patients underwent a neck dissection operation simultaneously to the primary tumour removal, and lymph node metastases were present in four cases. Eight patients underwent postoperative radiotherapy. Mean follow-up time (calculated in months from treatment completion to the last otolaryngological control) was 20.5 months (range 6-60 months). In the analysis of the clinical data, we defined poor oncological outcome as either recurrence of local disease or occurrence of metastasis after treatment. Clinicopathologic characteristics of the selected patients are shown in Table 1.

Immunohistochemistry

Forty specimens of formalin-fixed, paraffin-embedded tissue blocks were cut into 3-mm sections by a microtome. All specimens included samples originated from complete resection material. The slides were dewaxed, hydrated, and washed with TRIS-buffered saline. This process was followed by microwave treatment for 20 min in citrate buffer (pH= 6.0) to retrieve the antigens present. After blocking endogenous peroxidase activity in water with 3% H202 for 30 min, the tissue sections were incubated with anti-E-cadherin antibody (Clone NCH-38 diluted 1:50, DAKO, Denmark) for 30 min and then anti-mouse antibody for another 30 min. Immunodetection was performed with the Envision system, DAKO Autostainer, model VL1. Diaminobenzidine was applied for 10 min as a chromogen. The slides were then counterstained with hematoxylin. Appropriate positive and negative controls were included in all reactions.

Evaluation of E-cadherin expression

The slides were viewed randomly and without any clinical data by one of the authors. The staining was predominantly membranous with some cytoplasmatic staining also

	No. of patients	%
Sex		
Male	29	72.5
Female	11	27.5
T stage		
T1	12	30
T2	10	25
T3	16	40
T4	2	5
N stage		
N0	36	90
N1	3	7.5
N2	1	2.5
TNM stage		
I	12	30
II	8	20
III	18	45
IV	2	5
Histological grading		
G1	23	57.5
G2	17	42.5
Loco-regional recurrence		
L-R rec. no	32	80
L-R rec. yes	8	20

Table 1: Clinicopathologic characteristics of 40 patients with glottic squamous cell carcinoma

present. A staining score was given based on the percentage of cells stained (0–100%). All stained cells were considered positive regardless of the intensity of the staining.

STATISTICAL ANALYSIS

The correlations between the clinicopathologic parameters and the expression of E-cadherin were evaluated using the chi-square ($\chi 2$) test, the Fisher exact test and Kruskal-Wallis test. The role of each possible prognostic factor (univariate analysis) and the joint effect of all these factors (multivariate analysis) was explored using a multivariate logistic regression analysis. Disease-free survival analysis was based on the Kaplan-Meier method, and statistical significance was assessed by the log-rank test. To determine the effect of distinct prognostic factors on survival, a multivariate analysis was performed according to the Cox regression model. A p value less than 0.05 was considered to be significant in all statistical analyses. All statistical analyses were conducted with SPSS 13.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA).

RESULTS

Expression of E-cadherin was evaluated in 40 glottic laryngeal squamous cell carcinomas (29 male and 11 female). The age of the patients at diagnosis ranged from 44 to 81 years with a mean age of 63.2 years. Twenty patients had early cancer (Stage I or II) and 20 had advanced cancer (Stage III or IV).

Table 2: The correlation of E-cadherin expression with clinicopathologic parameters in patients with glottic squamous cell carcinoma

Variables	Number of patients	E-cadherin expression[a]		
		Low	High	p value[b]
Sex				1.0[c]
Male	29	17	12	
Female	11	7	4	
Age (years)				.792
? 59	16	10	6	
> 59	24	14	10	
T classification				.038
T1 and T2	22	10	12	
T3 and T4	18	14	4	
N status				.136
N0	36	20	16	
N+	4	4	0	
TNM stage				.010
I and II	20	8	12	
III and IV	20	16	4	
Histologic grade				.601
G1	23	13	10	
G2 and G3	17	11	6	
Loco-regional recurrence				.114
L-R rec. no	32	17	15	
L-R rec. yes	8	7	1	

a Low E-cadherin expression was below 27.35, High E-cadherin expression was above 27.35
b Chi-square ($\chi 2$) test or Fisher exact test

E-cadherin expression was associated with the cell membrane and varied greatly among tissue samples, with scores ranging from 2 to 72 (median 23). The mean expression score of E-cadherin in the considered glottic squamous cell carcinomas was 27.35 (standard deviation [SD] = 20.15). Using the mean expression of E-cadherin as a cut-off, 17 (42.5%) tumours were classified into the "high E-cadherin" group and the other 23 (57.5%) tumours were classified into the "low E-cadherin" group.

The correlations of E-cadherin expression with clinicopathologic parameters is summarised in Table 2. Decreased E-cadherin expression was significantly correlated with more aggressive tumours, including T3-T4-staged tumours (p= 0.038) and those with advanced clinical stage (TNM stage III and IV) (p= 0.010). There was no significant correlation between the expression of E-cadherin and age or sex. No relationship was observed between E-cadherin expression and histopathological differentiation (p= 0.601). Also, the Fisher exact test did not show any statistically significant difference in E-cadherin expression between pN+ and pN0/cN0 malignancies (p= 0.136).

The mean expression of E-cadherin was 38.5 (SD 22.49) in pT1 carcinomas, 24.5 (SD 19.54) in pT2 carcinomas, 22.75 (SD 16.92) in pT3 carcinomas, and 11.5 (SD 12.02) in pT4 carcinomas. We performed a non-parametric test for trends across order groups (a modified Kruskal-Wallis test) to identify differences in E-cadherin between pT stages, but no significant differences were shown (p= 0.140). The mean E-cadherin expression was 38.5 (SD 22.45) in stage I carcinomas, 25.63 (SD 21.97) in stage II carcinomas, 22.44 (SD 15.94) in stage III carcinomas and 11.5 (SD 12.02) in stage IV carcinomas. The differences in expression between the different TNM stages were not statistically significant (p= 0.140).

Eight of 40 patients with laryngeal squamous cell carcinomas developed loco-regional recurrence (3 local recurrences, 5 recurrences in the neck lymph nodes; mean expression of E-cadherin 17.63; median 18.5; SD 9.46). In the group without loco-regional recurrence, mean expression of E-cadherin was 29.78 (median 26; SD 21.45). Correlations of E-cadherin expression with tumour recurrence were not statistically significant (p=0.114).

The results of a stepwise logistic regression analysis showed that only the presence of lymph node metastasis was an independent predictor for tumour recurrence when grouping local and regional recurrences (odds ratio 18.6; p= 0.019; 95% CI, 1.601-216.056). There was a decline in disease-free survival associated with a decreased E-cadherin expression, but this was not significant (log-rank test p= 0.111). The presence of lymph node metastasis at the time of diagnosis (log-rank p= 0.002), age ≤ 59 years (log-rank p=0.003), and female gender (log-rank p=0.025) were all associated with worse disease-free survival. The results of a multivariate Cox proportional hazards model confirmed that the presence of cervical lymph node metastases (P=0.003) and age ≤ 59 years (P=0.006) were statistically significant independent predictors of a reduced disease-specific survival.

DISCUSSION

The presence of lymph node metastases is the single most adverse independent prognostic factor in head and neck squamous cell carcinoma (13). The preoperative detection of lymph node metastases is crucial to the effective treatment of these patients. Diagnostic techniques including computed tomography, magnetic resonance imaging, ultrasonography, positron emission tomography, and ultrasound-guided fine-needle aspiration biopsy have reached a sensitivity of more than 80% in detecting metastases (14), but they have the fundamental limitation that the metastases need to have a size of at least several millimetres to be detected. Thus, the ability to identify molecular markers from a primary tumour biopsy sample that can predict cervical lymph node metastases would enable the selection of patients at risk for lymph node metastasis. However, since invasion and metastasis are very complicated multistep processes, it is likely that more than one marker will be needed to assess an individual patient's risk of nodal metastases.

The process of metastasis is a cascade of linked sequential steps involving multiple host-tumour interactions. The suppression of cell-cell adhesiveness is accepted to have an important role in facilitating the dissemination of tumour cells from the primary site and the establishment of metastases (15). Among the several families of adhesion molecules, E-cadherin has been suggested to play a role in the process of nodal metastasis. E-cadherin, a calcium-dependent membrane protein, is essential for the formation of adheren junctions between cells (16). Catenins are involved in the regulation of cadherin function. Both β and γ catenins bind directly to the cytoplasmic portion of E-cadherin. α catenin plays a critical role in the transmembrane anchorage of the cadherins, and deletion of α catenin results in a non-adhesive cadherin/catenin phenotype. Downregulation of E-cadherin reduces cell-cell adhesion, reduces gap-junction mediated communication, and prevents terminal differentiation of cells, thus maintaining the ability to proliferate (17). The loss of E-cadherin expression in tumour tissue may lead to a more aggressive phenotype because neoplastic cells have a greater tendency to spread to adjacent tissues and lymph nodes. Abnormal expression of E-cadherin has been correlated in several human carcinomas with pathological characteristics of the tumour, such as tumour stage, invasiveness, lymph node involvement and distant metastases (18-21). Moreover, reduced expression of E-cadherin has been correlated with clinical variables, such as disease relapse and disease-free survival (22-24). Additionally, a negative correlation between E-cadherin expression and tumour differentiation has been observed. Well- or moderately-differentiated cancers preserve E-cadherin, while poorly differentiated ones express it poorly or not at all (10).

Studies of E-cadherin expression in squamous cell carcinomas of the head and neck have failed to provide a clear picture for its role in these tumours. Schipper et al. (12)

observed that E-cadherin expression decreased with loss of differentiation in primary carcinomas and that lymph node metastases expressed a lower level of the protein, suggesting an important role of cadherin loss in the metastatic process. In 1996, Franchi and colleagues (25) observed that low expression of E-cadherin in laryngeal squamous cell carcinomas significantly correlated with the presence of occult nodal metastases. Simionescu et al. (26) studied 42 cases of oral squamous cell carcinomas at different sites (tongue, lips, palate, and gums) and different grades of differentiation and investigated the immunoexpression of adhesion molecules. The study indicated a decreasing degree of immunostaining for E-cadherin parallel with decreases in oral squamous cell carcinoma differentiation grade. The loss of cell adhesion correlated to a decrease in E-cadherin expression, suggesting that E-cadherin may be a good prognostic predictor in oral squamous cell carcinoma evolution. Eriksen et al. (27) found that E-cadherin was strongly correlated with histopathological features associated with well-differentiated tumours and can be considered as a marker of differentiation in squamous cell carcinomas of the head and neck. Mattijssen et al. (11) also studied a group of 50 patients with head and neck squamous cell carcinoma. A relation between high levels of membrane-associated E-cadherin expression and favourable outcomes was found, although it did not reflect an absence of regional lymph node metastases. Liu et al. (28) studied markers associated with tumour invasion and metastasis in 59 patients with laryngeal and hypopharyngeal squamous cell carcinoma with node metastases. No relationship was found between the immunopositivity of cancer cells for E-cadherin and the presence of lymph node metastasis. Takes et al. (29) examined histological features and biological markers in 31 patients with laryngeal carcinomas. Of all markers investigated immunohistochemically, E-cadherin was not relevant to the prediction of lymph node metastasis.

In this study, we analysed the clinicopathologic significance of E-cadherin expression among 40 patients with laryngeal squamous cell carcinomas primary localised in glottic region. Our study found that lower expression of E-cadherin was significantly associated with a more aggressive tumour phenotype, including T3-T4 tumours and those with advanced clinical stage (TNM stage III and IV). There was no significant correlation between the expression of E-cadherin and age or sex. Generally, E-cadherin expression was found to be high in well differentiated cancers, but it was reduced in undifferentiated cancers (10-12, 25). Our results show a general, but not significant, decline in E-cadherin expression with increasing dedifferentiation of the tumour, a finding that is in line with those of Rodrigo et al. (30). Some studies have demonstrated a correlation between reduced E-cadherin expression and nodal metastases (12, 25), whereas others have failed to show this relationship (10, 29). In our study, the Fisher exact test did not show any statistically significant difference in expression of E-cadherin between pN+ and pN0/cN0 malignancies. The lack of statistical association between the nodal status and expression of E-cadherin was possibly due to the limited size of our preliminary series. In addition, we failed to find any significant association of E-cadherin expression with tumour recurrence.

CONCLUSION

Decreased expression of E-cadherin in primary glottic laryngeal squamous cell carcinomas correlated significantly with advanced T status and TNM stage. The results of the present study suggest that expression of E-cadherin may be useful to identify patients with more aggressive disease, allowing more effective treatment strategies to be implemented. Larger studies are required to confirm the role of E-cadherin expression in predicting the behaviour of laryngeal squamous cell carcinomas.

REFERENCES

1. Shimoyama Y, Hirohashi S, Hirano S, et al. Cadherin cell-adhesion molecules in human epithelial tissues and carcinomas. Cancer Res 1989; 49: 2128–33.
2. Veatch AL, Carson LF, Ramakrishnan S. Differential expression of the cell-cell adhesion molecule E-cadherin in ascites and solid human ovarian tumor cells. Int J Cancer 1994; 58: 393-9.
3. Chen HC, Chu RY, Hsu PN, et al. Loss of E-cadherin expression correlates with poor differentiation and invasion into adjacent organs in gastric adenocarcinomas. Cancer Lett 2003; 201 : 97-106.
4. Li Z, Ren Y, Lin SX, Liang YJ, Liang HZ. Association of E-cadherin and beta-catenin with metastasis in nasopharyngeal carcinoma. Chin Med J 2004; 117: 1232-9.
5. Sun W, Herrera GA. E-cadherin expression in urothelial carcinoma in situ, superficial papillary transitional cell carcinoma, and invasive transitional cell carcinoma. Hum Pathol 2002; 33: 996-1000.
6. Koksal IT, Ozcan F, Kilicaslan I, Tefekli A. Expression of E-cadherin in prostate cancer in formalin-fixed, paraffin-embedded tissues: correlation with pathological features. Pathology 2002; 34: 233-8.
7. Bohm M, Totzeek B, Birchmeier W, Wieland I. Differences of E-cadherin epression levels and patterns in primary and metastatic human lung cancer. Clin Exp Metastasis 1994; 12: 55-62.
8. Kanazawa T, Watanabe T, Kazama S, Tada T, Koketsu S, Nagawa H. Poorly differentiated adenocarcinoma and mucinous carcinoma of the colon and rectum show higher rates of loss of heterozygosity and loss of E-cadherin expression due to methylation of promoter region. Int J Cancer 2002; 102: 225-9.
9. Sarrio D, Perez-Mies B, Hardisson D, et al. Cytoplasmic localization of p120ctn and E-cadherin loss characterize lobular breast carcinoma from preinvasive to metastatic lesions. Oncogene 2004; 23: 3272-83.

10. Bowie GL, Caslin AW, Roland NJ, Field JKM, Jones AS, Kinsella AR. Expression of the cell-cell adhesion molecule E-cadherin in squamous cell carcinoma of the head and neck. Clin Otolaryngol 1993; 18: 196-201.
11. Mattijssen V, Peters HM, Schalkwijk L, et al. E-cadherin expression in head and neck squamous cell carcinoma is associated with clinical outcome. Int J Cancer 1993; 55:580-5.
12. Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K, Birchmeier W. E-cadherin expression in squamous cell carcinomas of head and neck. Inverse correlation with tumor differentiation and lymph node metastasis. Cancer Res 1991; 51: 6328-37.
13. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. N Engl J Med 2001; 345: 1890-900.
14. van den Brekel MWM, Castelijns JA, Snow GB. Diagnostic evaluation of the neck. Otolaryngol Clin North Am 1998; 31: 601-19.
15. Wijnhoven BPL, Dinjens WNM, Pignatelli M. E-cadherin-catenin cell-cell adhesion complex and human cancer. Br J Surg 2000; 87: 992-1005.
16. Guilford P. E-cadherin downregulation in cancer: fuel on the fire? Mol Med Today 1999; 5: 172–7.
17. Jongen WM, Fitzgerald DJ, Asamoto M, et al. Regulation of connexin 43-mediated gap junctional intercellular communication by Ca2+ in mouse epidermal cells is controlled by E-cadherin. J Cell Biol 1991; 114: 545–55.
18. Bukholm IK, Nesland JM, Karesen R, Jacobsen U, Borresen-Dale AL. E-cadherin and alpha-, beta-, and gamma-catenin protein expression in relation to metastasis in human breast carcinoma. J Pathol 1998; 185: 262-6.
19. Pignatelli M, Ansari TW, Gunter P, et al. Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. J Pathol 1994; 174: 243–8.
20. Shun CT, Wu MS, Lin JT, et al. An immunohistochemical study of E-cadherin expression with correlations to clinicopathological features in gastric cancer. Hepatogastroenterology 1998; 45: 944-9.
21. De Marzo AM, Knudsen B, Chan-Tack K, Epstein JI. E-cadherin expression as a marker of tumor aggressiveness in routinely processed radical prostatectomy specimens. Urology 1999; 53: 707-13.
22. Tamura S, Shiozaki H, Miyata M, et al. Decreased E-cadherin expression is associated with haematogenous recurrence and poor prognosis in patients with squamous cell carcinoma of the oesophagus. Br J Surg 1996; 83: 1608-14.
23. Gabbert HE, Mueller W, Schneiders A, et al. Prognostic value of E-cadherin expression in 413 gastric carcinomas. Int J Cancer 1996; 69: 184-9.
24. Charpin C, Garcia S, Bonnier P, et al. Reduced E-cadherin immunohistochemical expression in node-negative breast carcinomas correlates with 10-year survival. Am J Clin Pathol 1998; 109: 431-8.
25. Franchi A, Gallo O, Boddi V, Santucci M. Prediction of occult metastases in laryngeal carcinoma: role of proliferating cell nuclear antigen, MIB-1, and E-cadherin immunohistochemical determination. Clin Cancer Res 1996; 2: 1801-8.
26. Simionescu C, Mărgăritescu C, Surpățeanu M, et al. The study of E-cadherine and CD44 immunoexpression in oral squamous cell carcinoma. Rom J Morphol Embryol 2008; 49(2): 189-93.
27. Eriksen JG, Steiniche T, Søgaard H, Overgaard J. Expression of integrins and E-cadherin in squamous cell carcinomas of the head and neck. APMIS 2004; 112 (9): 560-8.
28. Liu M, Lawson G, Delos M, et al. Prognostic value of cell proliferation markers, tumor suppressor proteins and cell adhesion molecules in primary squamous cell carcinoma of the larynx and hypopharynx. Eur Arch Otorhinolaryngol 2003; 260: 28-34.
29. Takes RP, Baatenburg de Jong RJ, Schuuring E, et al. Markers for assesment of nodal metastasis in laryngeal carcinoma. Arch Otolaryngol Head Neck Surg 1997; 123: 412-9.
30. Rodrigo JP, Domínguez F, Alvarez C, Manrique C, Herrero A, Suárez C. Expression of E-cadherin in squamous cell carcinomas of the supraglottic larynx with correlations to clinicopathological features. Eur J Cancer 2002; 38(8): 1059-64.