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Original research-Orijinal araştırma

Do cytokeratins play a role as a prognostic biomarker in squamous cell carcinoma of larynx?

Sitokeratinler larenks yassı hücreli kanserinde prognostik belirteç olabilir mi?

M. Kürşat Gökcan, Alp Demireller, Özden Tulunay

Otorhinolaryngology, Head and Neck Surgery Department (M. K. Gökcan, M.D, Specialist, Prof. A. Demireller, M.D.), Pathology Department (Prof. Ö. Tulunay, M.D.) Ankara University Medical School, TR-06100 Ankara

Abstract

Aim. In this prospective study, expressions of distinct cytokeratin (CK) subtypes in larynx cancer (LC) were examined by immunohistochemistry (IHC) and these findings were correlated with known prognostic factors. **Method**. Thirty LC patients were enrolled into study. Tumor sections were examined and graded according to Broder's criteria. For examining the CK expression, polyclonal low molecular weight CK (LMWCK) and high molecular weight CK (HMWCK) antibody cocktails, and monoclonal CK18 and CK19 antibodies were applied into tumoral and non-neoplastic mucosa sections with streptavidin-biotin peroxidase technique. **Result**. Tumors included squamous cell (SC), adenosquamous cell, verrucous, basaloid carcinomas and sarcomatoid carcinoma. Grade 2 SC cancer was the most prevalent type (63.3 %). On IHC, LMWCK and HMWCK were found to have diffuse expression that disables any prognostic implication. CK-18 expression seemed to diminish by grade but to increase by stage. CK-19 expression had positive correlation with both grade and stage (p=0.064). **Conclusion**. This study is first research evaluating the correlation of CK staining findings with pathologic prognostic factors. Findings of this CK cocktail study suggest that these markers are not optimal for decision making of hyperplastic mucosa and differentiation degree in neoplasias, as they include a variety of CK classes that result in diffuse cytoplasmic staining. However with the addition of certain monoclonal CKs, CK-18 and 19 could have prognostic implications in SC carcinomas of larynx.

Key words: Larynx cancer, squamous cell carcinoma, tumor markers, cytokeratins, prognosis

Özet

Amaç. Larenks kanserinde belirli sitokeratin (SK) subtiplerinin immünhistokimya (IHK) ile ekspresyon derecelerine bakılarak sonuçların prognostik faktörlerle korelasyonu amaçlandı. **Yöntem.** Bu prospektif çalışmaya, 30 larenks kanseri olgusu dahil edildi. Tümör kesitleri histopatolojik olarak incelenerek Broder sistemine göre derecelendirildi. Ardından poliklonal yüksek molekül ağırlıklı SK (YMASK), düşük molekül ağırlıklı SK (DMASK) antikorları ve monoklonal SK 18 ve SK 19 antikorları streptavidin-biotin peroksidaz yöntemi ile tümöral ve non-neoplastik dokulara tatbik edilerek ekspresyon dereceleri incelendi. **Bulgular.** Tümörler yassı hücreli karsinoma (YHK), adenoskuamoz hücreli, verrüköz, bazaloid ve sarkomatoid karsinomlardan oluşmaktaydı. Grade 2 YHK en sık görülen tümör idi (% 63,3). IHK ile YMASK ve DMASK'ler herhangi bir prognostik incelemeye izin vermeyecek derecede yoğun ve yaygın boyandılar. SK-18 ekspresyon derecesi, tömörde grade artışı ile azalırken, evre artışı ile artmaktaydı. SK-19 ekspresyonunda ise hem evre, hem de grade artışı ile pozitif korelasyon izlendi. Prognostik değerlendirmede daha geniş bir seride SK-18 ekspresyonunun boyun metaztazı üzerine tahmin değeri bulunabileceği düşünüldü (p=0,064). **Sonuçlar.** Bu çalışma SK boyanma derecelerini patolojik prognostik faktörlerle ilişkilendirme açısından özgündür. Poliklonal SK'ler içerdikleri SK çeşitliliğinden dolayı farklılaşma derecesinin tayininde ve hiperplastik mukozaların değerlendirilmesinde uygun görülmemiştir. Ancak SK-18 ve 19, antikor paneline bazı SK antikorlar eklendiği takdirde larenks kanserinde prognostik değere sahip olabilir.

Anahtar sözcükler: Larenks kanseri, yassı hücreli karsinom, tümör belirteçleri, sitokeratinler, prognoz.

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Corresponding addres:

Dr. M. Kürşat Gökcan, Kulak Burun Boğaz Anabilim Dalı Ankara Üniversitesi Tıp Fakültesi TR-06100 Ankara, e-mail: gokcan@medicine.ankara.edu.tr

Introduction

According to the SEER Cancer Statistics Review of the National Cancer Institute, an estimated 12250 men and women will be diagnosed with larynx cancer (LC) in 2008; of those, 3670 will die [1]. Prognosis of the disease is known to be related with certain clinical parameters as laryngeal localization or stage of the disease and histological parameters like the differentiation of the tumor. As these clinical features can be unreliable predictors for individual patients and several features can only be investigated after surgical resection, it seems that there is need for further prognostic indicators for better evaluation of patients and for predicting treatment response [2].

Intermediate filament proteins (IFPs) are important markers of tissue differentiation and have been receiving increasing interest, in particular, through their applicability in the characterization of malignant tumors [3]. Cytokeratins (CKs) are a family of IFPs that are typically specific for epithelial cells. To date 20 subtypes of CKs have been found in human epithelia which are expressed in certain combinations depending on the type of the epithelium and the degree of differentiation [3]. As other cellular features, CK expression may be modified or lost during malignant transformation [4,5]. Previous studies demonstrated how the expression pattern of CKs in the normal laryngeal mucosa is altered in premalignant and malignant laryngeal lesions [3-6]. However the prognostic meaning of these changes in LC has not been clarified yet.

In an effort to find out possible predictors of biological behavior in LC, two separate pilot studies investigating IFP and neuroendocrine marker expressions were designed. In this study, CK marker expressions of LC specimens were studied with immunohistochemical analysis with a panel of monoclonal antibodies and different immunophenotypes were correlated with known clinical and histological prognostic parameters.

Material and Methods

Thirty patients operated and diagnosed as LC in our institutions were evaluated in this prospective study. The study included patients with squamous cell carcinoma (SCC) and its variants that were followed up for 5 to 38 months (mean 20.5 months), prospectively. None of the patients had been treated before surgery. The patients were staged according to the TNM system (6th Edition; UICC, International Union Against Cancer).

Histopathologic evaluation

All specimens related to laryngectomy and neck dissection were fixed and processed routinely. Hematoxylin and eosin (HE) stained slides were evaluated for diagnosis, growth patterns (i.e. ulcerous, papillary or keratotic), and surgical margins. Cellular and pathological properties of the tumors were recorded as follows:

Tumor border: Tumor front was evaluated as expansive or infiltrative and graded as 0 or 1 respectively.

Inflammatory reaction: Inflammatory reaction against tumor was graded as: none: 0,

scant around tumor: 1, scant in and around tumor: 2, moderate in and around tumor: 3, evident inflammation around each tumor foci: 4.

Tumoral stroma, desmoplasia: Desmoplasia was graded as: none: 0, scant: 1, moderate: 2, evident: 3.

Keratinization: Keratinization was graded as: none: 0, scant: 1, moderate: 2, evident: 3.

Pleomorphism: Pleomorphism of tumor cells were graded as; none: 0, scant: 1, moderate: 2, evident: 3

Mitotic index: Three fields that are rich of mitosis were chosen under x40 magnification, number of mitosis were count and mean value of these 3 fields were taken as mitotic index.

The scores of the abovementioned parameters were summed up for a "Total Score". A "Differentiation Score" was calculated by subtracting well differentiation score (keratinization) from the sum of poor differentiation scores (desmoplastic stroma, pleomorphism, mitosis and tumor border scores).

The tumors were graded according to the degree of differentiation; cellular pleomorphism, nuclear properties and mitotic index according to Broder's criteria as follows: well differentiated (grade 1; G1), moderately differentiated (grade 2; G2) and poorly differentiated (grade 3; G3) [7].

Mucosa adjacent to tumor was evaluated for the type of mucosa; stratified (SE), pseudostratified (PSE) or immature stratified epithelium (ISE), and for the mucosal changes according to the Ljubljana Classification [8].

Immunohistochemistry

Immunohistochemistry was performed using antibodies against low molecular weight cytokeratin (LMWCK), high molecular weight cytokeratin (HMWCK), CK-18 and CK-19. One paraffin-embedded tissue per case from a representative tumor area with adjacent non-tumoral mucosa was used for IHC. IHC analysis was evaluated on 4μm-thick sections mounted on to positively charged slides, and performed in an automatic immunohistochemistry staining machine (Benchmark-Ventana 320-202, Ventana Inc., Tuscon) by using streptavidin-biotin peroxidase method [9]. After dewaxing, slides were incubated with primary antibodies; LMWCK (Neomarkers; AE1, protease, 1/100), HMWCK (Neomarkers; AE3, protease, 1/100), CK-18 (Biogenex; MU1430496,Citrate under pressure and heat,1/100) and CK-19 (Zymed; A53-B/A2, Citrate, ready to use). Counter-stain was performed with Mayer's hematoxylin. Positive and negative controls were stained in parallel. The evaluation of IHC was based on intensity and distribution of the markers. Staining distribution of the markers was rated as: Rate 1 (0-5%), Rate 2 (6-25%), Rate 3 (26-50%), Rate 4 (51-75%), and Rate 5 (76-100%).

Statistical Analysis

For statistical evaluation, chi-square and Spearman's correlation tests were performed using Statistical Package for Social Sciences (SPSS), 16th version, SPSS Inc., Chicago, Il, USA).

Results

In this study 29 male, 1 female patient, aged between 37 and 70 years (mean age 56 years) were evaluated. Demographic data of patients, localization, stage of the tumors and the operations performed were listed at Table 1. Ten tumors were localized in glottis, 7 in supraglottis and 13 were transglottic region. With physical examination and radiologic evaluation, 20 patients were found to be N0 and 10 were N+. Tumors were grouped by stage as early stage (ES; Stage I and II), or advanced stage (AS; Stage III and IV) and 14 patients were at ES (46.7 %), 16 patients were at AS (53.3 %).

Table 1: Demographic data of patients, localization, TNM classification and stage of the tumors and treatment results are shown.

Case	Age	Location	T	N	M	Stage	Surgery	Neck dissection	Adjuvant RT	Result
1.	57	G	1	0	0	1	VPL	L- FND	No	NED
2.	42	SG	2	0	0	2	SHL	R- FND	No	NED
3.	59	G	2	0	0	1	LFC	None	Yes	NED
4.	61	TG	4	2B	0	4A	TL	R- MRND	Yes	NED
5.	54	TG	2	2C	0	4A	SHL	R- FND	Yes	Dead*
6.	63	G	1	0	0	1	FLL	None	No	NED
7.	69	TG	2	0	0	2	3\4 L	L- FND	No	NED
8.	47	G	1	0	0	1	LFC	NONE	Yes	NED
9.	58	TG	4	1	0	4A	TL	R- FND	Yes	NED
10.	62	TG	3	2B	0	4A	TL	L- RND	Yes	NED
11.	60	TG	3	1	0	3	TL	BIL FND	Yes	NED
12.	43	TG	3	0	0	3	TL	None	Yes	NED
13.	60	SG	2	1	0	3	SHL	L- MRND, R- FND	Yes	Dead**
14.	62	SG	2	0	0	2	SHL	R- FND	No	NED
15.	53	G	1A	0	0	1	LFC	None	Yes	NED
16.	64	G	3	0	0	3	TL	L- FND	Yes	NED
17.	70	G	2	0	0	2	VPL	None	No	NED
18.	59	TG	2	0	0	2	VPL	None	No	NED
19.	65	G	1	0	0	1	LFC	None	No	NED
20.	60	G	2	0	0	2	VPL	None	No	NED
21.	58	TG	3	0	0	3	TL	None	Yes	NED
22.	63	TG	4	2C	0	4A	TL+PP	R- RND	Yes	NED
23.	64	SG	2	2C	0	4A	TL	R- FND	Yes	NED
24.	47	SG	2	2	0	4A	3\4 L	R- FND	Yes	NED
25.	43	TG	3	0	0	3	TL	BIL FND	No	NED
26.	50	G	2	1	0	3	TL	R- FND	No	NED
27.	37	SG	1	0	0	1	SHL	BIL FND	No	NED
28.	53	TG	3	0	0	3	TL	L- FND	Yes	NED
29.	41	TG	2	0	0	2	SCPL	None	No	NED
30.	53	SG	4	0	0	4A	TL	BIL FND	No	NED

*: Disesase specific death,**:Disease non-related death, NED: No evidence of disease) (Except subject 7, all subjects were male) (G: Glottic, S: Supraglottic, T: Transglottic, L: Left, R: Right, BIL: Bilateral, VPL: Vertical partial larygectomy, SHL: Supraglottic horizontal laryngectomy, LFC: Cordectomy with laryngofissure, ¾ L: ¾ laryngectomy, SCPL: Supracricoid partial laryngectomy, TL: Total laryngectomy, FND: Functional neck dissection, (M)RND: (Modified) Radical neck dissection ,PP: Partial pharyngectomy

Laryngectomy specimens included 13 totals, 17 partial laryngectomies. Histopathologic evaluations of specimens were outlined at Table 2. On microscopic evaluation G2 SCC was the most common tumor (63.3 %). Other specimens included 3 G1 SCC (10 %), 1 G3 SCC (3.33 %), 3 G1 verrucous carcinoma (VC) (10 %), 2 G3 Sarcomatoid carcinoma (SC) (6.67 %), 1 (3.33%) undifferentiated basaloid Ca (BSCC) and 1 (3.33%) adenosquamous Ca (ASC).

Table 2. Histopathologic findings.

Case	Pathology/ Grade	Surgical border invasion	Cartilage invasion	Subglottic invasion	Extra laryngeal invasion	MET/ LAP
1	2	-	-	-	-	0
2	2	+	-	-	-	0
3	1	-	-	-	-	0
4	2	-	+	+	+	1 (R)
5	2	-	-	-	-	6 (R),1(L)
6	SC	-	-	-	-	0
7	SC	-	-	-	-	0
8	2	+	-	-	-	0
9	2	-	-	-	+	2 (R)
10	2	-	-	+	-	0
11	2	-	-	-	-	3 (R),1 (L)
12	ASC	-	-	-	-	0
13	2	-	-	-	-	2 (L)
14	2	-	-	-	-	0
15	1	+	-	-	-	0
16	2	+	+	+	+	0
17	2	-	-	-	-	0
18	2	-	-	-	-	0
19	2	-	-	-	-	0
20	VC	-	-	-	-	0
21	3	+	+	+	+	0
22	1	-	+	-	+	5 (R)
23	2	-	-	-	-	1 (R)
24	BSSC	+	-	-	-	1 (R)
25	VC	-	+	+	+	0
26	2	-	-	+	-	0
27	2	-	-	-	-	0
28	2	-	-	-	-	0
29	2	-	-	-	-	0
30	VC	-	+	-	+	0

L: Left, R: Right,SCC: Squamous Cell Carcinoma, MET: Metastases, LAP: Metastatic lymph node number, SC: Sarcomatoid Carcinoma, VC: Verrucous Carcinoma, ASC: Adenosquamous Carcinoma, BSCC: Basaloid SCC

Five patients that had positive surgical margin(s) on pathological evaluation were given adjuvant radiotherapy (RT). Subglottic invasion was found in 6 total laryngectomy (TL) specimens, 2 of them had also surgical margin positivity and were given adjuvant RT. Seven patients required extended TL due to extralaryngeal tumor spread. Five of those were given adjuvant RT, 2 of them were chosen for wait and see as they had VC and the tumors were believed to be removed totally.

Differentiation scores are given in Table 3. Evaluation of tumor fields under high magnification, revealed that keratin pearl and single cell keratinization rich tumors are mostly seen at well differentiated tumors (p: 0.006). However there were also examples where this feature was seen together with poorly differentiated tumors with high mitoses, cellular pleomorphism and infiltrating tumor border. Interestingly, keratinization degree was found to have positive correlation with thyroid cartilage infiltration (p: 0.016) and

extralaryngeal spread (p: 0.04) parameters in our series.

TABLE 3: Differentiation and immunohistochemistry scores.

Case	Keratin	Pleomorphism	Mitosis	Tumor border	Desmoplasia	Inflammation	Total score	Poor DS	DS	CK-18	CK-19	AE1	AE3
	0	3	6	0	0	1	9	9	9	0	0	3	2
	0	3	9	1	0	2	13	13	13	1	4	1	5
	3	0	0	1	0	2	4	1	-2	1	1	5	5
	3	0	6	1	1	1	11	8	5	0	2	2	2
	1	1	4	1	0	2	7	6	5	0	5	1	5
	1	2	14	0	0	0	17	16	15	0	1	5	1
	2	0	0	0	0	1	2	0	-2	1	5	5	1
	0	2	6	1	1	3	9	9	9	N/A	N/A	3	2
	1	1	1	1	1	2	4	4	3	1	1	N/A	N/A
	3	0	0	0	0	2	3	0	-3	1	1	5	5
	2	1	1	0	0	2	4	2	0	5	5	5	5
	0	2	6	1	1	1	9	9	9	3	5	5	5
	0	1	3	0	0	2	4	4	4	N/A	N/A	5	5
	2	2	5	0	0	2	9	7	5	0	5	5	5
	2	0	2	1	0	1	5	3	1	0	1	5	5
	1	2	3	1	1	2	7	7	6	0	0	5	5
	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	4	5	5
	1	0	4	0	0	2	5	4	3	1	5	5	5
	2	1	2	1	1	2	6	4	2	1	4	N/A	N/A
	3	0	1	0	0	2	4	1	1	0	1	5	5
	3	1	1	1	1	0	6	3	0	0		5	5
	2	2	10	1	1	1	15	13	11	2	1	5	5
	1	2	8	0	0	1	11	10	9	2	5	5	5
	0	3	11	1	1	0	14	18	18	N/A	1	N/A	4
	3	0	0	1	1	2	4	1	-2	1	0	5	5
	2	0	3	1	1	1	6	4	2	1	5	5	5
	2	3	4	1	0	1	10	8	6	0	4	5	5
	1	0	3	1	0	3	5	4	3	0	1	5	5
	2	1	6	0	0	2	9	7	5	0	1	5	5
	3	0	0	1	0	3	3	1	-2	2	1	5	5

Keratin (0-3), Pleomorfism (Pleo.) (0-3), Mitosis (n/x40 magnification), Tumor Border (0= expansive, 1= infiltrative), Inflammation (Infl.) (0-3) and desmoplasia (Desm.) (0-3). D.S: Differentiation Score, CK: Cytokeratin, AE1: Low molecular weight CK cocktail, AE3: high molecular weight CK cocktail

Neck dissection was performed for 19 patients of whom 10 were N+ clinically (Tables 1 and 2). Eight of them were found have neck metastasis with extracapsular spread and treated with adjuvant RT. Of those, one developed local recurrence and lost due to the disease (Case 5). Other recurrence in this series was seen after open cordectomy procedure and treated with RT (Case 3). He received chemotherapy for colon cancer on the postoperative 26th month and had no evidence of local or distant recurrence due to laryngeal cancer on follow up.

As a result, on 5-38 months follow up (mean 20.5 months), 2 patients had local recurrence and one was lost due to disease. One more patient was lost on postoperative

20th month due to atherosclerotic heart disease, without evidence of LC recurrence.

Immunohistochemical features

Results of streptavidin-biotin peroxidase IHC staining results with AE1, AE3, CK18 and CK19 are shown in Table 3.

As the subject number (30 patients) and follow up period (mean 20.5 months) were limited for statistical analysis, survival analysis for the prognostic meaning of IHC findings could not be performed. Results were interpreted by correlating and cross tabulating them with the clinical and histopathological parameters.

LMWCK and HMWCK results

Among 30 subjects, CK cocktails, AE 1 (LMWCK; CK10,13,14,15,19) was studied in 27 and AE3 (HMWCK; CK1,2,3,4,5,6,7,8) was studied in 28 of them. Both AE1 and AE3 showed similar expression patterns with similar intensities. Normal laryngeal epithelium showed scattered suprabasal expression which was more evident in hyperplastic epithelium and this pattern was also seen in differentiated tumor cells (Figure 1 A, B, C). Though LMWCK expression seemed to decrease with increased tumor grade (Figure 1 D), examples of increased tumor grade with keratinization showed diffuse immunostaining. Both markers were found to have strongly positive and similar expression levels (r: 0.4; p: 0.03) in all cases without any meaningful stage or grade difference.

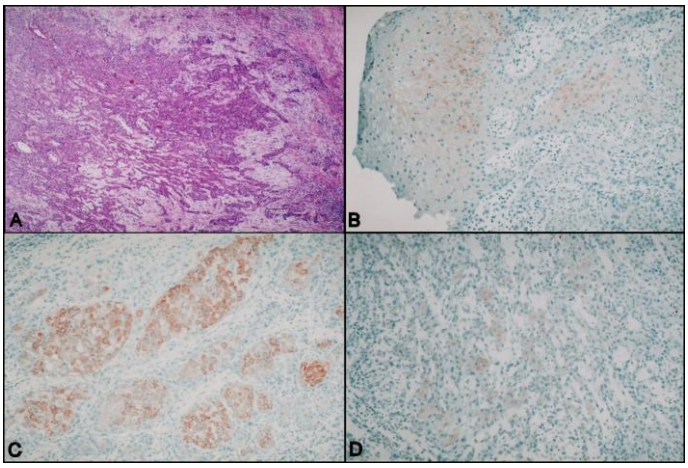

Figure 1 A. An example of a grade 2 squamous cell carcinoma with infiltrating borders into desmoplastic stroma (H&E, x 10). B. Tumor shows AE1 expression in suprabasal cells in hyperplastic mucosa and in spinous like cells in the invasion front (Peroxidase, x25). C. Better differentiated areas show diffuse cytoplasmic AE1 expression (Peroxidase, x25). D. Less differentiated areas show scattered AE1 expression (Peroxidase, x25).

Cytokeratin 18 (CK- 18)

No expression was observed in normal laryngeal mucosa, PSE and ISE sections. Of the 26 LC cases that CK 18 has been studied, 12 cases (46.1 %) showed weak positivity (level 1 and 2) and only 2 (7.7 %) showed stronger (level 3 and 5) expression. This expression was more apparent in G1 tumors as scattered heterogeneous positivity (Figure 2 A and B) which turned out to be scarce with decrease in differentiation (Figure 2 C and D).

CK 18 showed higher expression levels on advanced stage (stage III and IV) patients than early stage (stage I and II) patients (p: 0.04). Negative correlation between CK-18 staining and grade level (r:-0.237, p: 0.2) was seemed to be going together with the pleomorphism (r:-0.194, p: 0.35) and the number of mitosis (r:-0.263, p: 0.2), but did not reach to significance.

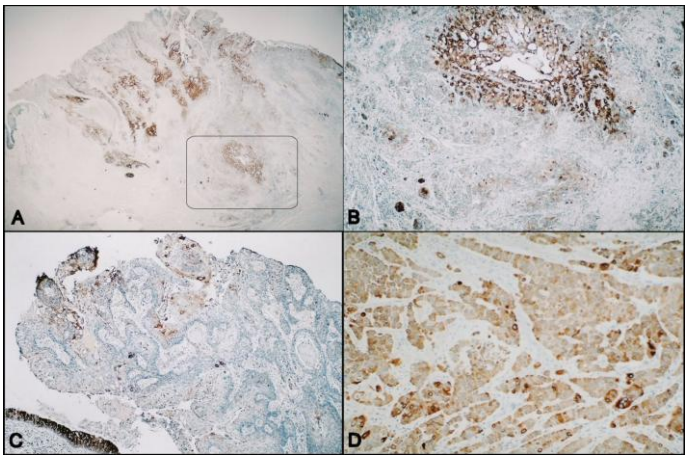

Figure 2 A. An example of a squamous cell carcinoma that shows heterogenous CK-18 expression (Peroxidase, x 2.5). B. Closer view (inset) of the CK-18 expressing island on the tumor base (Peroxidase, x10). C. A less keratinized tumor showing scattered (Rate: 1) CK-18 expression in the superficial keratinized areas (Peroxidase, x10) D. Another grade 2 squamous cell carcinoma showing heterogeneous and weak CK-18 expression in infiltrating areas (Peroxidase, x10).

Cytokeratin 19 (CK-19)

Normal larynx mucosa sections in our series showed interrupted basal cell expression. Differentiation of normal mucosa to hyperplasia, atypical hyperplasia and in situ carcinoma revealed an expression that included whole basal layer and extended to suprabasal layers in varied amounts accordingly (Figure 3 A and B). This pattern was also continued according to tumor grade; CK-19 expression increased with increased tumor grade (Figure 3 C and D).

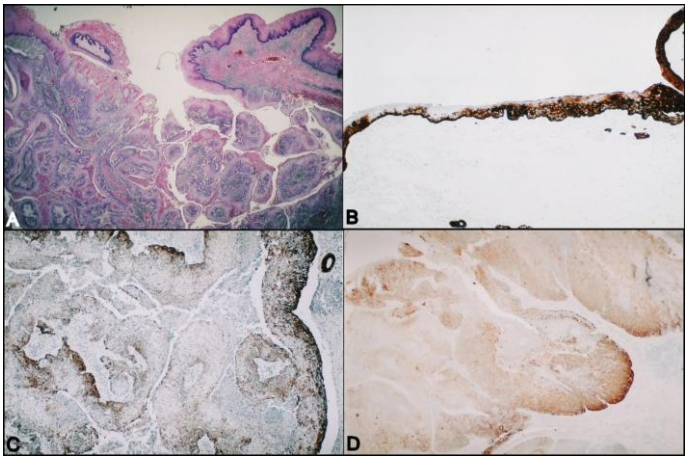

Figure 3 A. An example of a grade 2 squamous cell carcinoma showing papillary organization in ulcerous tumor base (H&E, x2.5). B. CK-19 expression in basal cell layer that extends to whole cell layer in atypical hyperplastic areas. Neighbouring glands and pseudostratified epithelium are also strongly CK-19 positive (Peroxidase, x10). C. Better differentiated areas show less CK-19 expression (Peroxidase, x10). D. Less differentiated areas show better CK 19 expression (Peroxidase, x25).

The expression of CK 19 according to stage showed positive correlation between CK-19 expression level and increase in stage though that was near but has not reached to statistical significance (r:0.757, p:0.06). Among other clinical parameters, CK-19 expression was negatively correlated with cartilage invasion (r:-0.418, p: 0.03) and extralaryngeal tumor invasion (r:-0.469, p: 0.016).

Discussion

In this study, CK expression pattern in normal, hyperplasic laryngeal mucosa and LC of different stage and grade were studied with both polyclonal CK cocktails (AE1 and AE3) and monoclonal CK-18 and 19 antibodies. As results could not be correlated with survival function, significance of IHC expression levels were interpreted by correlating and cross tabulating them with the known clinical prognostic parameters as tumor location and stage and histopathological parameters as grade, keratinization, pleomorphism, inflammation and desmoplasia levels.

Histopathologic criteria as tumor border status, desmoplasia, inflammation and keratinization are known to effect clinical prognosis. Having an infiltrating tumor border is a worse prognostic indicator compared to having expansive tumor border [10, 11]. In our series, cases that had infiltrating tumor border, also showed significant desmoplasia, thyroid cartilage infiltration and extralaryngeal infiltration. Inflammatory response in and around tumor seemed to decrease desmoplasia and increase tumor differentiation in our histopathologic evaluation.

Cytokeratin cocktails (AE1 and AE3) showed scattered suprabasal expression which was more evident in hyperplastic epithelium and differentiated tumor cells. With decrease in tumor differentiation, there were examples of both lower and higher levels of CK cocktail staining depending on the level of keratinization (Figure 1). Other works also defined similar staining pattern that covers all stratified squamous epithelium except basal layer and increases in intensity in superficial layers [12, 13]. In our series, AE1 and AE3 resulted in rate 5 cytoplasmic expression in most of the cases (81.5 % and 78.6 % respectively) which hindered statistical correlation and cross tabulation of results with prognostic factors (Tables 2 and 3). On the other hand, Mallofre et al [13] studied AE1 and AE3 expression in a larger series of 50 patients. They evaluated the degree of immune staining as diffuse (> 50 %), mild (10-50 %) and weak (< 10%) and degree of differentiation as low grade and high grade. In a larger series with broader groups, their Chi square tests revealed significant relation between AE1 and AE3 staining density and clinical parameters as T stage, N stage and degree of differentiation. But the result of our CK cocktail study suggests that these markers are not optimal for estimating prognosis in LC, as they include a variety of CK classes that result in diffuse cytoplasmic staining.

CK-18 and 19 are markers that are not expressed in normal laryngeal epithelium and their expression is accepted as an early molecular finding in malignant transformation [6, 12]. CK-18 is a CK that is synthesized mainly by simple epithelia and embryonic epithelia [3]. Our study also showed no CK-18 expression in normal laryngeal epithelium. Among LC sections, CK 18 expression was observed in 14 cases that were more evident in G1 cases as scattered heterogeneous positivity which turned out to be scarce with decrease in differentiation (Figure 2). Van der Velden et al. [12] also studied CK-18 expression in 29 LC specimens and, contrary to our results, found increase in staining intensity with increase in the tumor grade. Expression was reported to be especially abundant in tumors with a basaloid cell phenotype, and in ten cases expression increased towards the tumor margins. There were 13 N+ cases in their series that were all positive for CK-8 and 18. Similarly, in our series we have found an association between neck metastases and CK-18 expression that could reach statistical significance with increased subject number (p: 0.064). We assume that CK-18 positivity might have a positive predictive value on neck metastases in a larger series.

CK-19 is found in a broader range of epithelial tissues. It appears as a major component in many simple epithelia and usually as a minor component in diverse stratified epithelia as well as squamous cell carcinoma cells [3]. In our LC series, CK-19 expression was increased with increase in tumor grade and stage. In Val der Velden et al. [12] study, CK-19 expression was also found to be sparse in low grade tumors and increase in immune reactivity in all layers of tumor cells were seen with increased tumor grade.

As a result, both CK-18 and 19 were found to have increased expression with advance in disease stage that reached statistical significance with CK-18. Upon comparison with similar studies, we realized that grading, staging and immune reactivity scoring systems in those studies were not unique and studies that used CK cocktail antibodies [5, 13] may sometimes have different results with the studies that used monoclonal antibodies [6].

Conclusion

In this preliminary study with limited series and follow up period, we could not estimate prognostic meaning of studied CK's with survival function. However we believe that CK-18 and 19 would be promising biomarkers in laryngeal cancer, with the addition of stratification markers CK-4 and 13, basal cell marker CK-17, simple cell markers CK-7 and 8 and cornification marker CK-10 to antibody panel.

Conflict of Interest: The authors declare that they have nothing to disclose financially during conduction of the study.

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