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Original scientific paper

Peritumoral p53 Expression in Oral Carcinoma

Faris Fočo[1], Nurija Bilalović[2], Semir Vranić[2], Fadila Serdarević[3], Irma Ramović[1] and Edin Imamović[1]

[1] Clinical Center of the University of Sarajevo, Department of Maxillofacial Surgery, Sarajevo, Bosnia and Herzegovina
[2] Clinical Center of the University of Sarajevo, Department of Pathology, Sarajevo, Bosnia and Herzegovina
[3] Sarajevo University School of Medicine, Departments of Epidemiology and Medical Statistics, Sarajevo, Bosnia and Herzegovina

A B S T R A C T

p53 is one of the most frequently mutated genes in human tumors including head and neck tumors like oral squamous cell carcinoma. It might be responsible for more than 50% of all relapses in patients with surgically treated oral carcinoma and clean margins. The aim of the present study was to explore p53 protein expression in peritumoral tissue and correlate it with relapse of the disease. The study included 25 patients (17 males and 8 females) with oral squamous cell carcinoma in the period August 2006 till August 2008. For immunohistochemical assay, a monoclonal antibody against p53 protein was applied (clone DO-7, DAKO Glostrup, Denmark). Peritumoral expression of p53 was as follows: 10 out of 25 cases (40%) were negative, 2 cases (8%) showed weak, 5 cases (20%) moderate and 8 cases (32%) strong p53 positivity. No significant correlation between peritumoral expression of p53 protein and patient's relapse was found. In contrast, we found a trend toward association between intratumoral p53 expression and patient's relapse (p=0.07). There was also trend toward higher peritumoral p53 expression in females comparing with p53 expression in males (52.9% of males did not have p53 expression while 87.5% females had mild, moderate or high p53 expression, p=0.088). Peritumoral expression of p53 protein is frequently seen in oral squamous cell carcinoma and merits further research.

Key words: *oral carcinoma, squamous cell carcinoma, p53 protein, survival, relapse*

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer for both sexes in the general population and the third most common cancer in developing countries with more than 350,000 new cases worldwide every year[1].*P53* gene is the most frequently mutated gene in human tumors[2]. Disruption of the p53 signaling pathway is also one of the most common genetic alterations of the head and neck tumors [1,3]. Its product, p53 protein, plays a fundamental role in the control of cell proliferation, apoptosis and genetic stability[1, 4]. The prognostic role of p53 protein in OSCC has already been established with several studies that pointed out p53 as a prognosticator of poor outcome though there are studies that could not confirm its prognostic value[3,5,6]. Several studies pointed out that there was no significant difference in p53 protein expression in relation to different oral cavity anatomical sites of OSCC[7]. It is noteworthy that p53 is also important predictor of radiotherapy response in patients with OSCC[8,9] Peritumoral expression of p53 in oral carcinoma is poorly understood with very few studies that specifically focused on the p53 expression in peritumoral normal mucosa and its correlation with outcome[10, 11]. Early local recurrence is one of the main causes of treatment failure of OSCC contributing significantly to low survival rates in these patients[12]. Also, clear surgical margins can not predict local recurrence in a significant proportion of patients with OSCC. Therefore we wanted to explore peritumoral expression of p53 and its correlation with relapse as well as other clinicopathologic parameters in OSCC.

Materials and Methods

Clinical data

This prospective study included 25 patients (17 males and 8 females), diagnosed with squamous cell carcinoma of oral cavity in the period August 2006 till August 2008. All clinicopathologic data are summarized in Table 1. The control examinations were performed each 6, 12, 18 and 24 months. The last follow-up data were obtained in December 2008.

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TABLE 1
CLINICAL, HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL FEATURES OF 25 PATIENTS WITH OSCC

Characteristic	Number of patients (%)
Age (mean, range)	65.88 (49–92)
Sex	Males: 17 (68%)
	Females: 8 (32%)
Tumor stage	pT1: 6 (24%)
	pT2: 17 (68%)
	pT3: 2 (8%)
Lymph node status	No: 21 (84%)
	N1: 2 (8%)
	N2: 2 (8%)
Metastasis	Mo: 23 (92%)
	M1: 2 (8%)
Tumor grade	G1: 9 (36%)
	G2: 15 (60%)
	G3: 1 (4%)
Peritumoral p53 protein expression	Negative: 10 (40%)
	Weak: 2 (8%)
	Moderate: 5 (20%)
	Strong: 8 (32%)
Smoking	Smokers: 13 (52%)
	Non-smokers: 12 (48%)
Alcohol consumption	Yes: 3 (12%)
	No: 22 (88%)
Relapse of the disease	Yes: 6 (24%)
	No: 19 (76%)

Histopathology and Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues of 25 primary oral squamous cell carcinomas were used for routine diagnosis. The tumor grading was done according the current WHO classification of the tumors of head and neck (2005). The tumor staging was done using the 7th American Joint Committee on Cancer Recommendations (2010). Immunohistochemical assay for p53 protein was done using a monoclonal antibody (clone DO-7, DAKO, Glostrup, Denmark). p53 protein positivity was estimated as follows: < 10% positive cells (negative), 11–40% positive cells (weak), 41–70% (moderate) and > 71% positive cells (strong positivity).

Statistical analysis

For correlation analysis Fischer exact test was used. One-way ANOVA and post-hoc tests were applied to compare means. A computer program package for social sciences (SPSS 11.5, Chicago, IL, USA) was used for all statistical testing. The statistical significance was accepted for $p<0.05$.

Results

Clinico-pathologic characteristics of the cohort are summarized in Table 1. All tumors were oral squamous cell carcinomas (lip and oral cavity) (Figure 1) of which 9 (36%) were low grade whereas 15 (60%) were intermediate and 1 (4%) high grade. Significantly high tumor grades were found among females than among males ($p=0.032$, Fischer's exact test, Table 2). Similarly, a higher percentage of females were in stage pT2 compared with males ($p=0.022$, Fisher's exact test). During the follow-up period 6 patients (24%) had relapse of the disease (2 females and 4 males). Peritumoral expression of p53 was as follows: 10 out of 25 cases (40%) were negative, 2 cases (8%) showed weak, 5 cases (20%) moderate and 8 cases (32%) strong p53 positivity (Table 3, Figure 2A-D). Intratumoral p53 protein expression had the following distribution: Five (20%) out of 25 tumors showed negative staining of p53 protein whereas 11 (44%) showed weak, 4 (16%) moderate and 5 (20%) strong p53 protein overexpression. We did not find a significant correlation between peritumoral expression of p53 protein

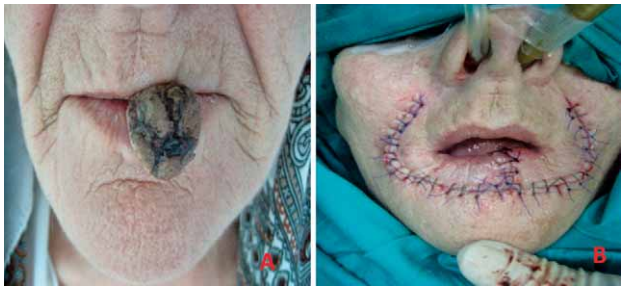

Fig. 1. A patient with squamous cell carcinoma before sugery (a) and after surgery (b).

TABLE 2
SIGNIFICANTLY DIFFERENT DISTRIBUTION OF THE TUMOR GRADE BETWEEN GENDERS

Sex	Grade 1	Grade 2	Grade 3	Total
Male	8 (32%)	8 (32%)	1 (4%)	17 (68%)
Female	0	8 (32%)	0	8 (32%)
Total	8 (32%)	16 (64%)	1 (4%)	25 (100%)

TABLE 3
DISTRIBUTION OF PERITUMORAL P53 PROTEIN EXPRESSION ACCORDING TO PATIENT'S GENDER

p53 expression	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	Total
Male	10 (40%)	1 (4%)	2 (8%)	4 (16%)	17 (68%)
Female	0	1 (4%)	3 (12%)	4 (16%)	8 (32%)
Total	10 (40%)	2 (8%)	5 (20%)	8 (32%)	25 (100%)

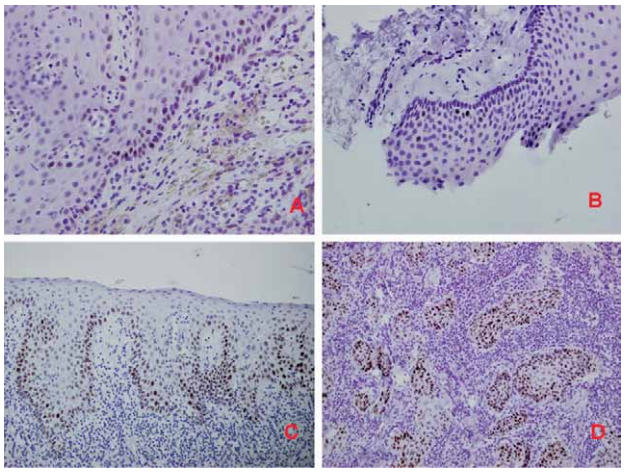

Fig. 2. Illustrating various expressions of p53 protein in peritumoral tissue.

and patients' relapse. In contrast, we found a trend of correlation between intratumoral p53 expression and patient's relapse (p=0.07). There was also trend toward higher peritumoral p53 expression in females comparing with p53 expression in males (52.9% of males did not have p53 expression while 87.5% female tissue samples showed p53 expression, p=0.088). We found also significant correlation between p53 expression and patient's age (p=0.031). No other significant correlation between the variables was found.

Discussion

Peritumoral p53 protein expression was detected in 52% of OSCC analyzed in our study. This is in accordance with other studies that also demonstrated high p53 protein positivity in surgical margins although these were previously morphologically stated to be clean[11]. Several studies have already pointed out that clear surgical margins could not predict the relapse of the disease in a significant percentage of OSCC cases. This discrepancy may be caused by p53 alterations in the peritumoral tissue as our results also indicate (see Table 4). However, we could not find a significant association between peritumoral p53 expression and local recurrence. This can be partially explained by a small sample size and a relatively short follow-up of the patients included in our study although the most local recurrences occur within two first years after the initial diagnosis. Unlike other studies, intratumoral expression of p53 protein was not high as expected since only 36% of the cases showed moderate and strong nuclear p53 expression. The most studies found p53 protein overexpression in OSCC in approximately 50% of the cases[7,13–16]. The elucidation of such finding might be that we had a relatively small percentage of high grade tumors and the most tumors were in its early stage (pT1 and pT2). Also, such a discrepancy can be explained by other factors including the methodology employed, type of tumor material and heterogeneity of tumoral sites examined in our study. We also could not confirm statistically significant association between tumor expression of p53 protein and relapse of the disease although there was a trend toward a significant association (p=0.07). It is noteworthy that the prognostic value of the tumor p53 expression is somewhat contradictory although more studies confirmed p53 as a prognosticator of poor outcome[5, 17].

TABLE 4
A SUMMARY OF THE KEY CORRELATION FINDINGS

Variables	n (%)	n (%)	p-value
Tumor size	Size 1	Size 2–3	
Positive p53 expression on mucosal margins	2 (33.0)	0	0.05
Tumor grade	Grade 1	Grades 2–3	
Positive p53 expression on mucosal margins	2 (25.0)	0	0.093
Tumor relapse	No relapse	Relapse	
Positive p53 expression in tumor cells	17 (89.5)	3 (50)	0.07

Peritumoral p53 protein expression showed significant positive relationship with the tumor size since the larger tumors showed higher p53 expression. It is not surprising since the loss of p53 gene function is related to increased cell proliferation and tumor neoangiogenesis[18]. However, it is not well understood how peritumoral cells influenced the tumor growth although the relationship between the tumor stroma and tumor cells has been established and well documented[19].We also want to point out that high percentage of the patients included in the study were smokers (Table 1). Smoking is a well established risk factor not only for oral carcinoma but also for several other malignant and non-malignant conditions[20] though clear relationship between smoking and p53 mutation has not been established yet[21]. This public health problem needs to be resolved as soon as possible since it dramatically might reduce the incidence of OSCC as the third most common cancer in developing countries[1]. This is particularly important if we know that overall five-year survival in patients with OSCC was among the lowest with an average of 54% in the United States[21]. There is no relevant data about survival rate of the patients in Bosnia and Herzegovina but it probably is lower than in the United States.

Conclusion

In summary, there was no significant correlation between peritumoral expression of p53 and relapse whereas we found a trend toward correlation between intratumoral p53 expression and relapse of the disease. Peritumoral p53 expression was significantly associated with the tumor size. Although small sample size in our study did not allow revealing statistically significant association between p53 expression within tumor cells and cancer relapse, further research involving larger sample size is warranted.

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F. Fočo

Clinical Center of the University of Sarajevo, Department of Maxillofacial Surgery, Hazima Šabanovića 1, 71000 Sarajevo, Bosnia and Herzegovina
e-mail: farisfoco@yahoo.com

PERITUMORSKA EKSPRESIJA P53 KOD KARCINOMA USNE ŠUPLJINE

SAŽETAK

P53 je jedan od najčešće mutiranih gena kod tumora, uključujući tumore kao što je planocelularni karcinom glave i vrata. Mogao bi biti odgovoran za više od 50% relapsa kod bolesnika kod kojih je tumor odstranjen kirurški, uz negativne rubove. Cilj ovog istraživanja je odrediti ekspresiju p53 u peritumorskom tkivu i usporediti sa relapsima bolesti. Studija je uključivala 25 bolesnika (17 muških i 8 ženskih) sa planocelkularnim karcinomom u razdoblju od kolovoza 2006 do kolovoza 2008. Monoklonalno protutijelo protiv p53 je upotrebljavano za imunohistokemijsku analizu (clone DO-7), DAKO Glostrup, Denmark). Peritumorska espresija p53 je bila slijedeća: 10 od 25 slučajeva su bili negativni (40%), 2 su pokazala slabu ekspresiju (8%), 5 umjerenu (20%), a 8 jaku ekspresiju p53 (32%). Nije nađena značajna korelacija između peritumorske p53 ekspresije i relapsa. Nasuprot tome, našli smo povezanodst između intratumoralne p53 ekspresije i relapsa (p=0.07). Također je postojala viđa peritumoralna ekspresija p53 kod žena prema muškarcima. Peritumoralna ekspresija p53 se često vidi kod karcinoma usne šupljine i zaslužuje daljnja istraživanja.