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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

p16 status of oropharyngeal and oral cavity squamous cell carcinomas – a single institution experience

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SUMMARY

Introduction/Objective New World Health Organization Classification of Head and Neck Tumors from 2017 has introduced significant changes, mainly considering tumors in the oropharyngeal region. New entities of HPV-positive and -negative squamous cell carcinomas have been acknowledged, not only based on the presence of an active viral infection and different tumor markers expression, but also because of their different histopathology, staging assessment, and prognosis. A retrospective study has been conducted, in order to determine p16 positivity in squamous cell carcinomas in oropharynx and in the oral cavity, and to see whether they differ in sex and age distribution.

Methods The presence of viral infection was verified based on p16 immunochemistry staining, p16 being the surrogate marker for HPV infection. A total of 177 cases of squamous cell carcinomas in the oropharynx and the oral cavity, found in the archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Centre of Serbia, have been revised.

Results Out of 177 cases, 50 (28.2%) were p16-positive. Compared with carcinomas in the oral cavity, p16 carcinomas were significantly more common in the oropharynx (34.3% in the oropharynx, compared to 10.3% in the oral cavity). Carcinomas in both regions were mostly associated with male sex (88.1% of all cases were in males), but p16 positivity was more common in females (11 out of 21 cases, 52.4%). The most common location of p16-positive carcinomas were palatine tonsils (41.03% of tonsillar carcinomas were p16-positive).

Conclusion P16-positive squamous cell carcinomas were the most numerous in the oropharynx, i.e. palatine tonsils, and were more common in females.

Keywords: oropharynx; oral cavity; human papilloma virus; squamous cell carcinoma

INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC) is a medical problem on the rise. In the new World Health Organization (WHO) classification from 2017, oropharyngeal tumors are found to be so important that they are separated in a new chapter, being divided from tumors in the oral cavity, in which they were incorporated in previous classification from 2005 [1, 2]. These tumors are now classified as p16-positive, associated with *human papillomavirus* (HPV) infection, and p16-negative, associated with long-term cigarette smoking and alcohol abuse [3–7].

Oral HPV infection has been proven as a significant factor in the genesis of squamous cell carcinoma (SCC) in this region [4]. Infection is usually transmitted by urogenital sexual contact, so it is mostly found in young, sexually active adults [4, 5]. In the past few years, there has been a dramatic increase in the incidence of OPSCC attributed to HPV infection, mainly in developed countries [8, 9, 10]. Most common viral subtype found in these tumors is HPV 16, which has a high tumorous potential [5, 11].

P16-positive SCC originates from malignant transformation of the oropharyngeal epithelium, caused by viral replication within the cells. Most convenient for HPV replication is tonsillar crypt epithelium, so generally these carcinomas occur most commonly in the palatine tonsils. Cancer grows by imitating tonsillar crypt architecture, so it differs from p16-negative carcinomas not only by etiology, but also by histopathology [1, 2, 5]. Given that the cancer arises not from the superficial squamous epithelium but from the reticulated epithelium which coats the tonsillar crypts, most of the cancers are non-keratinizing and of basaloid appearance [2, 5].

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Consequently, grading of tumors into well differentiated, poorly differentiated, and undifferentiated is no longer applicable, so the new WHO Classification discourages the practice of grading HPV-positive OPSCC. Ultimately, p16-positive OPSCC have been related to a significantly better prognosis in comparison to p16-negative OPSCC, as well as to a lower risk of cancer recurrence [12, 13, 14].

There is no specific test for proving the active HPV infection in the oropharynx, so expression of the p16 protein in cells is used as a reliable surrogate marker [5, 15]. p16 is a tumor suppressor protein that inhibits cyclin-dependent kinase 4A, which further phosphorylates the retinoblastoma (Rb) protein and allows the cell to progress from G1 to S phase of the cell cycle. By a feedback mechanism, Rb normally inhibits the expression of p16. Carcinogenesis of most of the head and neck carcinomas involves inactivation of the tumor suppressor proteins, including p16. However, viral E7 protein produced in HPV infection leads to functional inactivation of the Rb protein, and consequential overexpression of the p16 protein, which can be used to detect these tumors by immunohistochemical staining methods [5, 15].

The aim of the study is to determine p16 positivity of SCC in the oropharynx and the oral cavity, as well as to determine differences in age and sex distribution.

METHODS

A retrospective study has been conducted based on cases found in archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery (Clinical Centre of Serbia), from January 1, 2013 to October 1, 2017. A total of 173 cases of SCC in the oropharynx and the oral cavity were found, as well as four cases of primarily found metastasis of this carcinoma in the neck lymph nodes.

All the samples were standardly processed in the Clinic's laboratory (formalin-fixed, paraffin-embedded and cut into 4 μm sections), and sent for staining to the Immunohistochemistry Laboratory of the Institute of Pathology, Faculty of Medicine, Belgrade. A monoclonal p16 antibody was used (anti-mouse, ready-to-use; Ventana Medical Systems, Inc., Oro Valley, AZ, USA). Samples were stained partly manually, partly in an immunostainer (BenchMark Special Stains, Ventana Medical Systems, Inc.) following the standard laboratory protocols. Detection involved a two-stage UltraVision LP detection system (Thermo Fisher Scientific, Waltham, MA, USA), which included secondary (Primary Antibody Enhancer) and tertiary (large volume HRP polymer) antibodies. The reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride chromogen. The samples were then stained with hematoxylin according to standard protocols and evaluated under a microscope. Samples with both nuclear and cytoplasmic immunoreactivity of more than 75 % of tumor cells were considered p16-positive (Figure 1). All histopathological procedures including tissue manipulations were a part of a routine diagnostic procedure that is applied for our patients.

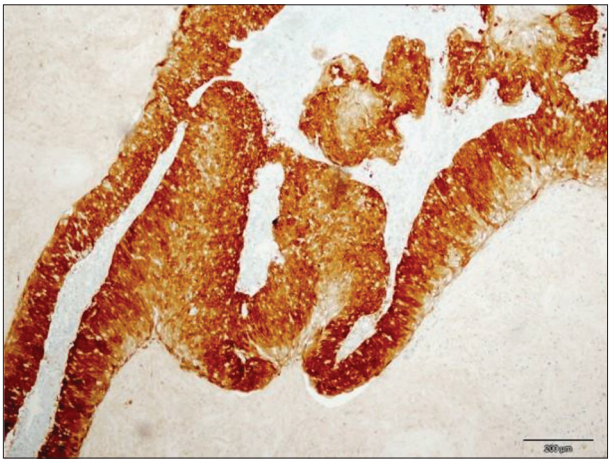

Figure 1. An example of p16-positive staining sample: intensive nuclear and cytoplasmic positivity in more than 75% of tumor cells (streptavidin-biotin, × 400)

The data was processed in IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) using the methods of descriptive and analytic statistics. Based on the type of data, the following tests have been used: Fisher's exact test, χ^2 test, ANOVA, student's t-test. The results were considered significant for p-values less than 0.05.

The paper was approved by the institutional review board.

RESULTS

Out of 177 patients with SCC, 156 (88.1%) were male and 21 (11.9%) were female. The average age was 62.82 ± 10.19 years; the youngest patient was 28, and the oldest one 93 years old. The most common localization of all SCC was the palatine tonsil, in 78 (44.1%) patients. Other most common localizations were as follows (Figure 2): the tongue base (14.1%), the mobile tongue (11.3%), and the posterior pharyngeal wall (11.3%).

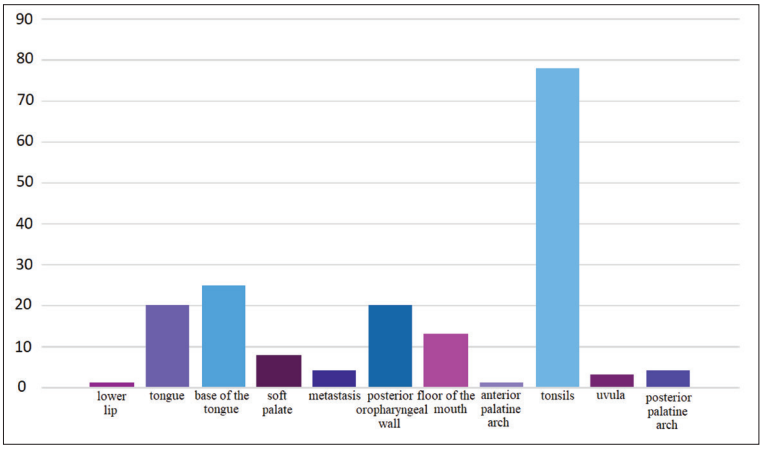

Figure 2. Site distribution of squamous cell carcinomas in the oropharynx and the oral cavity

All the cases were divided in three groups. The first group (Table 1) were OPSCC, which included tumors in the tonsils, the soft palate, the tongue base, the posterior oropharyngeal wall, and the uvula (n = 134). The second group (Table 2) consisted of the oral cavity SCC, including tumors on the lips, floor of the mouth, the mobile tongue, the anterior and posterior palatine arches (n = 39). The third group was made out of four cases of primarily found metastasis of SCC in the lymph nodes of the neck region (n = 4).

The groups were compared regarding the p16 positivity, age and sex distribution (Tables 1 and 2). Sex distribution among the groups was not significantly different (p = 0.179), and neither was age distribution (p = 0.541). p16 positivity was most commonly found in the oropharynx (34.3% of the cases); in the oral cavity, 10.3% of SCC were p16-positive, whereas all four of the metastases were p16-negative. Statistically significant difference was found in the p16 positivity distribution among the groups (p = 0.006, Table 3). Out of all p16-positive SCC, 92% were in the oropharynx and only 8% in the oral cavity.

In the oropharynx, there were 46 p16-positive cases, out of which 37 (80.4%) were male and nine were female (19.6%). The average age of p16-positive patients was 64.83 ± 10.68 years. The other 88 cases were p16-negative, with 84 males (95.5%) and four females (4.5%), and the average age was 62.41 ± 7.8 years.

In total, there were 50 (28.2%) p16-positive cases in both regions. Out of all males, 39 (25%) had p16-positive SCC, whereas 11 (52.4%) females had p16-positive SCC. P16 positivity was significantly more common in females (p = 0.009, Table 4). The mean age of p16-positive patients was 65.12 ± 10.71 years, and the mean age of p16-negative patients was 61.92 ± 9.87 years. p16-positive and -negative patients did not significantly differ in the age distribution (p = 0.06).

Table 1. Distribution of squamous cell carcinoma in various parts of the oropharynx

Localization	Frequency
Tonsils	78 (58.2%)
Root of the tongue	25 (18.7%)
Soft palate	8 (6%)
Posterior oropharyngeal wall	20 (14.9%)
Uvula	3 (2.2%)

Table 2. Distribution of squamous cell carcinoma in the oral cavity

Localization	Frequency
Lower lip	1 (2.6%)
Tongue	20 (52.3%)
Floor of oral cavity	13 (33.3%)
Palatoglossal arch	1 (2.6%)
Palatopharyngeal arch	4 (10.3%)

Table 3. Distribution of p16-positive and negative squamous cell carcinoma in the oropharynx and the oral cavity (p = 0.006, χ^2 test)

p16 status	Oropharynx (n = 134)	Oral cavity (n = 39)
p16+	46 (34.3%)	4 (10.3%)
p16-	88 (65.7%)	35 (89.7%)

Table 4. Distribution of p16 positivity as to sex (p = 0.009, χ^2 test)

p16 status	Male (n = 156)	Female (n = 21)
p16+	39 (25%)	11 (54.4%)
p16-	117 (75%)	10 (47.6%)

DISCUSSION

P16-positive OPSCC have been acknowledged as new entities based on their significant differences in regard to p16-negative SCC in this region. Correlation with HPV infection causes their predominant occurrence in the palatine tonsils and their characteristic histological appearance, lower mean age of patients affected, and better prognosis and lower recurrence rates in comparison to SCC related to smoking and alcohol abuse [1, 2, 5–9, 12–16].

In our study, an expected higher incidence of p16-positive SCC in the oropharynx, followed by the oral cavity, has been shown (34.3% in the oropharynx compared to 10.3% in the oral cavity). However, in our population, p16-negative SCC prevailed, in contrast to the USA, Sweden, and the Netherlands [8, 9, 10]. Given that these are more developed countries, leading the anti-smoking campaign for many years, it is understandable why there is a significant fall in the incidence of p16-negative SCC in the last few decades. On the other hand, more liberal sexual behavior could explain higher incidence of HPV infection in these countries.

Considering the lack of data on incidence of p16-positive SCC in our country prior to 2013, we could not determine whether there has been a serious increase in its incidence. A study conducted in the USA showed an increase in the incidence of p16-positive SCC of 225%, in the 1988–2004 period, whereas a study from Sweden determined a rise of 295% from the 2000–2002 period to the 1970–1979 period [8, 9]. Meta-analysis done based on data from Europe and North America showed a significant rise in the number of p16-positive SCC, from 47.7% before 2000 to 72.2% in the 2005–2009 period [16].

The mean age of patients with p16-positive OPSCC in our study did not significantly differ from the mean age of patients with p16-negative carcinomas, though p16-positive patients were on average older than the p16-negative ones (64.83 ± 10.67 years for positive, and 62.41 ± 7.87 years for negative), which is different from statistics in other countries. Studies conducted in the USA and Sweden concluded that patients with p16-positive SCC were significantly younger than those with p16-negative SCC, which is expected given the risk factors [8, 9].

Both p16-positive and -negative SCC were found significantly more often in males (90.3% of all cases were male, and only 9.3% were female), which is in accordance with other studies [8, 9, 10].

p16 positivity in our study was more often found in females; 69.23% of the females had p16-positive OPSCC, in comparison to 30.58% of the males. This information correlates with study conducted in Stockholm, where 96% of the females had a p16-positive carcinoma, in comparison to 81.58% of the males [9].

In sites other than oropharynx and oral cavity, routine p16 immunostaining is not recommended [17]. In selected patients with enlarged level II/III lymph nodes,

p16 immunohistochemistry may be considered since HPV-associated OPSCC often present with large cervical metastases with occult primary carcinoma.

CONCLUSION

p16-positive SCCs are most common in the oropharynx, namely in the palatine tonsils, and are more commonly found in females.

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Conflict of interest: None declared.

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Статус *p*16 сквамоцелуларних карцинома орофаринкса и усне дупље – искуство наше институције

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САЖЕТАК

Увод/Циљ Нова класификација тумора главе и врата Светске здравствене организације из 2017. године унела је значајне промене, углавном везане за регију орофаринкса. Уведени су нови ентитети *HPV* позитивних и негативних сквамоцелуларних карцинома, не само на основу присуства активне вирусне инфекције и експресије различитих туморских маркера већ и због своје различите хистопатологије, процене стадијума болести и прогнозе. Спроведена је ретроспективна студија са циљем да се одреди *p*16 позитивност у сквамоцелуларним карциномима орофаринкса и усне шупљине, као и како би се утврдило да ли постоји разлика међу различитим половима и старосним групама.

Методе Присуство вирусне инфекције је потврђивано на основу имунохистохемијске анализе на *p*16, који је маркер за инфекцију хуманим вирусом папилома. Увидом у архиву Патохистолошке лабораторије Клинике за оториноларингологију и максилофацијалну хирургију Клиничког центра Србије прегледано је 177 случајева сквамоцелуларних карцинома орофаринкса и усне шупљине.

Резултати Од 177 случајева, 50 (28,2%) случајева је било *p*16 позитивно. У поређењу са карциномима усне шупљине, карциноми *p*16 су били значајно чешћи у орофаринксу (34,3% у орофаринксу у поређењу са 10,3% у усној шупљини). Карциноми у обе регије су били чешћи код припадника мушког пола (88,1% свих случајева били су мушког пола), али је *p*16 позитивност била знатно чешћа код женских болесника (11 од 21 случаја, 52,4%). Најчешћа локализација *p*16 позитивних карцинома били су непчани крајници (41,03% карцинома крајника било је *p*16 позитивно).

Закључак *p*16 позитивни сквамоцелуларни карциноми били су најбројнији у орофаринксу (тачније у непчаним крајницима) и чешћи су били код женског пола.

Кључне речи: орофаринкс; усна шупљина; хумани вирус папилома; сквамоцелуларни карцином