Prevalence of Human Papillomavirus in Laryngeal, Oropharyngeal and Oral Cavity Carcinomas in Zadar County, Croatia

Prevalencija humanog papiloma virusa kod karcinoma larinksa, orofarinksa i usne šupljine u Zadarskoj županiji

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Abstract

We present a retrospective research of HPV testing of tissue samples, by the polymerase chain reaction method (PCR), in patients that underwent surgery due to squamous cell carcinoma of the oral cavity, oropharynx or larynx in Zadar General Hospital in the period from 2007 to 2016.

Out of 99 patients included in this study, 89 (89,9%) were men with median age of 62.3 years and ten (10,1%) were women with median age of 66 years. According to the cancer site, the percentage of HPV positive tissue samples of larynx carcinoma, oral cavity carcinoma and oropharyngeal carcinoma were 25.8%, 26.9% and 18.2%, respectively. According to the HPV genotype, among all cancer sites, in 60% (n=15) HPV 16 were detected, and in 40% mixed HPV infection, that is, in 32% (n=8) HPV 16, 26, 34, 53 and in 8% (n=2) HPV 16, 18, 26, 34, 53.

The presented research results support the need for stronger public health promotion of vaccines against HPV, and the selection of the best strategy for the implementation of vaccination to prevent cancer of the larynx, oropharynx and oral cavity among men.

Sažetak

Radi se o retrospektivnom istraživanju HPV testiranja metodom lančane reakcije polimeraze (PCR) uzoraka tkiva pacijenata operiranih zbog karcinoma skvamoznih stanica usne šupljine, orofarinksa ili grkljana u Općoj bolnici Zadar u razdoblju od 2007. do 2016. godine.

Od 99 pacijenata uključenih u ovu studiju, 89 (89,9%) bili su muškarci s medijanom dobi od 62,3 godine i 10 (10,1%) su bile žene s medijanom dobi od 66 godina. Ukupno je u 25 (25,3%) uzoraka tkiva bolesnika otkrivena prisutnost HPV DNA. Prema mjestu raka, postotak uzoraka HPV pozitivnih karcinoma tkiva, karcinoma grkljana, karcinoma usne šupljine i karcinoma orofaringeusa bio je 25,8%, 26,9% odnosno 18,2%. Prema genotipu HPV-a, od svih sijela karcinoma, u 60% (n=15) otkriven je HPV 16, a u 40% mješovite HPV infekcije, i to u 32% (n=8) HPV 16, 26, 34, 53 te u 8% (n=2) HPV 16, 18, 26, 34, 53.

Predstavljeni rezultati istraživanja podupiru potrebu jače javnozdravstvene promocije cjepiva protiv HPV-a, te odabir najbolje strategije za provedbu cijepljenja za prevenciju raka grkljana, orofarinksa i usne šupljine kod muškaraca.
Introduction

Squamous cell carcinoma (SCC) are the most common types of head and neck (H&N) cancers. H&N cancers accounted for an estimated number of 140,000 new cases and 63,500 deaths in Europe in 2012, about 4% of all cancers arising in Europe\(^1\). About 91% of all H&N cancer are squamous cell carcinomas, 2% are sarcomas and the other 7% are adenocarcinomas, melanomas and not well defined tumours\(^2\). H&N cancers are mainly associated with tobacco and alcohol use\(^3\). Other known risk factors are human papillomavirus (HPV)\(^4\) and Epstein-Barr virus (EBV) infections\(^5\), and low consumption of fruit and vegetables\(^6\). The major etiological role of HPV in oropharyngeal squamous cell carcinoma (OPSCC) has been proved since 2011.

Despite declining smoking prevalence in Europe\(^7\), the incidence rates of epithelial cancers of nasopharynx, hypopharynx and larynx remained quite stable, while those of oropharynx and oral cavity increased\(^8\). Rate of cancers associated to human papillomavirus (HPV) among H&NCs is highly depending on world region and tobacco use prevalence. It increases in high-income countries. In the United States (US) population, the number of HPV-associated oropharyngeal cancers now exceeds the number of cervix cancers\(^9\). More than 70% of OPSCC cases and over 50% of tonsillar cancers in the USA have been associated with high-risk HPV types\(^10\). In Europe, persistent infections with oncogenic HPV account for around 73,000 anogenital and 14,000 oropharyngeal cancer cases per year\(^10\). Studies have shown that oral sexual activities as well as an increase in the number of oral sexual partners increase oral transmission of HPV\(^6\). Possible routes of virus transmission include contact of the oral region with the infected anogenital region, autoinoculation, genital HPV transmission, vertical mother-to-child transmission, and horizontal transmission\(^15\). An increase in oral transmission of HPV consequently leads to an increase in the infection of the head and neck region; a higher number of HPV infections of the head and neck region has been reported in men compared to women\(^11-13\). This is probably due to a higher number of men giving oral sex to HPV-infected partners. Thus, oral sex is associated with most cases of HPV infection of the head and neck region. Oral cavity carcinomas most often occur in the horseshoe-shaped area of the oral cavity and in about 40% in the tongue area, 30-35% at the bottom of the oral cavity, 13% in the retromolar triangle and about 10% affects the gingiva and buccal mucosa\(^14\). Considering the ability of viruses to cause malignant changes in the epithelium of human they are divided into three groups: HPV high risk type: 16, 18, 31, 33, 35, 45, 52, 56, HPV low risk type: 6, 11, 26, 30, 42, 43, 44, 53, 54, 55 and HPV medium risk type: 31, 33, 35, 39, 51, 52\(^15\). HPV infections, among other things, are associated with HNCs. Prophylactic immunisation against HPV provides an opportunity for cancer control, as HPV vaccines have proven to be highly efficacious when given before a person becomes sexually active. However, in Europe, HPV vaccine uptake among preadolescent girls has remained far below the target levels in many countries\(^16\). Around 19 types of viruses have been proved to be in the head and neck carcinoma of which HPV 16 is the most frequent, followed by HPV 18, 33, 35, 45, 52\(^17\). The aim and purpose of this research was to investigate the presence and types of HPV in carcinomas of the oral cavity, oropharynx and larynx, in patients treated in the Department of Otorhinolaryngology (ORL) of Zadar General Hospital, in the ten-year period from 2007 to 2016.

Examinees and methods

This retrospective observational research was based on the medical documentation of Zadar General Hospital. The presence of HPV has been analysed in patients that underwent surgery due to OPSCC (ICD-10 C01- C14) at the Department of Otorhinolaryngology of Zadar General Hospital in the period of ten years. The Department of Pathology of Zadar General Hospital preserves the slides and paraffin blocks of all the patients that have undergone surgery. The mentioned slides of patients that underwent surgery due to squamous cell carcinoma of the larynx, oral cavity and oropharynx in the described period have been set aside and examined by a specialist pathologist. Paraffin blocks were set aside of those patients with confirmed squamous cell carcinoma. Seven incisions 10 micrometres thick were taken from each one. These seven incisions were placed in a xylene tube to remove the paraffin by centrifugation for two minutes at 15-25°C. Ethanol was then added (96-100%) to remove the xylene and centrifuged at room temperature for two minutes. They were then left at 37°C for 10 minutes for the ethanol to evaporate. Following DNA isolation with QIA amp DNA FEPE Tissue Kit and Gene Proof HPV, PCR Kit 50, PCR method, HPV was detected in the cancer. DNA extraction was performed by QIA amp DNA FEPE Tissue Kit following the manufacturer’s instructions.

Gene Proof Human Papillomavirus PCR Kit is designed for the detection of 24 high-risk types of Human Papillomavirus (HPV is indicated in the HEX fluorophore fluorescence channel) and typization of
Results

A total of 99 patients had undergone surgery due to carcinomas of the larynx, oral cavity and oropharynx at the Department of Otorhinolaryngology of Zadar General Hospital in the period of 10 years. Of the total number, 89 (89.9%) patients were male. Altogether 62 (62.6%) patients had undergone surgery due to squamous cell carcinoma of the larynx, 26 (26.3%) patients had undergone surgery due to squamous cell carcinoma of the oral cavity, while 11 (11.1%) patients had undergone surgery due to squamous cell carcinoma of the oropharynx (Table 1).

Of the 99 patients that have undergone surgery due to squamous cell carcinoma of the oral cavity and oropharynx, 25 (25.3%) were positive for HPV (Table 2). Of the 62 patients that had undergone surgery due to larynx carcinoma, 16 (25.8%) were positive for HPV, and among the 26 that had undergone surgery due to oral cavity carcinoma, seven (26.9%) were positive and among the 11 patients that have undergone surgery due to oropharynx carcinoma, two (18.2%) patients were positive for HPV (Tables 2 and 3).

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### Table 1. Number of patients treated due to head and neck cancer in Zadar General Hospital in the period of ten years, by sex and cancer location

<table>
<thead>
<tr>
<th>Cancer location</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larynx</td>
<td>56 (90.3)</td>
<td>6 (9.7)</td>
<td>62 (62.6)</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>22 (84.6)</td>
<td>4 (15.4)</td>
<td>26 (26.3)</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>11 (100)</td>
<td>0</td>
<td>11 (11.1)</td>
</tr>
<tr>
<td>All locations</td>
<td>89 (89.9)</td>
<td>10 (10.1)</td>
<td>99 (100)</td>
</tr>
</tbody>
</table>

### Table 2. HPV status of cancer tissue samples in patients treated due to head and neck cancers in Zadar General Hospital in the period of ten years

<table>
<thead>
<tr>
<th>Cancer location</th>
<th>HPV+ (%)</th>
<th>HPV - (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larynx (n=62)</td>
<td>16 (25.8)</td>
<td>46 (74.2)</td>
</tr>
<tr>
<td>Men</td>
<td>14 (87.5)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>2 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Oral cavity (n=26)</td>
<td>7 (26.9)</td>
<td>19 (73.1)</td>
</tr>
<tr>
<td>Men</td>
<td>6 (85.7)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Oropharynx (n=11)</td>
<td>2 (18.2)</td>
<td>9 (81.8)</td>
</tr>
<tr>
<td>Men</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>All locations (n=99)</td>
<td>25 (25.3)</td>
<td>74 (74.7)</td>
</tr>
<tr>
<td>Men</td>
<td>22 (88.0)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>3 (12.0)</td>
<td></td>
</tr>
</tbody>
</table>
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This significant proportion of HPV in carcinomas of the larynx, oral cavity and oropharynx coincides well with other studies, where 20.6% of HPV-positive tumours worldwide were found, 21.6% in North America, 21.5% in Europe [20]. Among Jordanian patients, the highest proportion of HPV was with oropharyngeal carcinoma 41.7%, oral cavity 37% and larynx 18.2% [21]. Likewise, global research in Italy indicated that the proportion of HPV positive was 30% with oropharyngeal tumour, but that it was on the rise and the dominant type was 16 [24], while authors in South Europe came to similar results by following that group of tumour [22]. The significance of this study is that this is the first research in Croatia of this type and so far there have been no scientific epidemiological data on the prevalence of HPV infection in carcinoma of the larynx, oral cavity and oropharynx.

Globally, there are roughly 96,000 incident OPSCC cases/year of which 20–24% are caused by HPV. Of these cases, 80–90% are due to HPV16 infection and would be prevented with the presently registered HPV vaccines. In Western countries, such as Sweden (with almost 400 TSCC and BOTSCC cases per year) and the United States, HPV prevalence in OPSCC is higher and around 70% [19]. Among Jordanian patients, the highest proportion of HPV was with oropharyngeal carcinoma 41.7%, oral cavity 37% and larynx 18.2% [21]. Likewise, global research in Italy indicated that the proportion of HPV positive was 30% with oropharyngeal tumour, but that it was on the rise and the dominant type was 16 [24], while authors in South Europe came to similar results by following that group of tumour [22]. The significance of this study is that this is the first research in Croatia of this type and so far there have been no scientific epidemiological data on the prevalence of HPV infection in carcinoma of the larynx, oral cavity and oropharynx. Globally, there are roughly 96,000 incident OPSCC cases/year of which 20–24% are caused by HPV. Of these cases, 80–90% are due to HPV16 infection and would be prevented with the presently registered HPV vaccines. In Western countries, such as Sweden (with almost 400 TSCC and BOTSCC cases per year) and the United States, HPV prevalence in OPSCC is higher and around 70% [19].

Of the 14 HPV positive male patients who had undergone surgery due to squamous cell carcinoma of the larynx, nine (64.3%) were HPV positive for type 16, four (28.6%) patients were HPV positive for type 16, group 26/34 and 53, and only one (7.1 %) patient was HPV positive for type 16, 18, group 26/34 and 53. Of the two female HPV positive laryngeal carcinoma patients, one (50%) was positive for type 16 HPV and one (50%) for type 16, group 26/34 and 53 (Table 3).

Out of seven HPV positive patients that had undergone squamous cell carcinoma of the oral cavity, three (42.9%) were positive for type 16 HPV, three (42.9%) for type 16, group 26/34 and type 53 HPV and only one (14.2%) for type 16, 18, group 26/34 and type 53 HPV. Out of six HPV positive male patients with oral cavity cancer, two (33.3%) had HPV type 16, three (50%) HPV type 16, group 26/34 and type 53 and one (16.7%) HPV type 16, 18, group 26/34 and type 53 HPV. In one HPV positive female patient that had undergone surgery of oral cavity carcinoma, HPV type 16 was established (Table 3).

Out of 11 patients that had undergone surgery due to squamous cell carcinoma of the oropharynx, that is, cancer of the base of the tongue and tonsils, two (18.2%) were positive for the presence of HPV, and two (100%) for type 16. (Table 3).

**Discussion**

In 2007, HPV type 16 was finally recognized as a risk factor, besides smoking and alcohol, for oropharyngeal squamous cell carcinoma (OPSCC), including tonsillar squamous cell carcinoma (TSCC), by the International Agency for Research against Cancer [19].

In this study we have observed that the largest proportion of HPV is found in laryngeal carcinoma (25.25%), followed by oral cancer (24%) and oropharyngeal cancer (12%). This significant proportion of HPV in carcinomas of the larynx, oral cavity and oropharynx coincides well with other studies, where 20.6% of HPV-positive tumours worldwide were found, 21.6% in North America, 21.5% in Europe [20]. Among Jordanian patients, the highest proportion of HPV was with oropharyngeal carcinoma 41.7%, oral cavity 37% and larynx 18.2% [21]. Likewise, global research in Italy indicated that the proportion of HPV positive was 30% with oropharyngeal tumour, but that it was on the rise and the dominant type was 16 [24], while authors in South Europe came to similar results by following that group of tumour [22]. The significance of this study is that this is the first research in Croatia of this type and so far there have been no scientific epidemiological data on the prevalence of HPV infection in carcinoma of the larynx, oral cavity and oropharynx. Globally, there are roughly 96,000 incident OPSCC cases/year of which 20–24% are caused by HPV. Of these cases, 80–90% are due to HPV16 infection and would be prevented with the presently registered HPV vaccines. In Western countries, such as Sweden (with almost 400 TSCC and BOTSCC cases per year) and the United States, HPV prevalence in OPSCC is higher and around 70% [19]. We have, therefore, established that the most common types were 60% (16), types 16, 26, 34, 53 in 32% (8), and in 8% (two) patients types 16, 18, 26, 34, 53. This is quite similar to studies that found that HPV 16, 18 and 35 were most common in squamous cell carcinoma of the head and neck, and had a longer survival after treatment [24-26]. According to the type of HPV virus, in 15 (60%) patients the HPV type was 16, which is similar to other studies. It is interesting that research proved a mixed infection with high-risk types and group 26/34 and 53 that belong to low-risk types (32%) (8) of positive cancers were with types 16, group 26/34 and type 53 HPV, and

<table>
<thead>
<tr>
<th>HPV types</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>16,53, group 26/34*</td>
</tr>
<tr>
<td>Larynx</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>All locations</td>
<td>15 (60%)</td>
</tr>
</tbody>
</table>

*Group 26/34 mean: Multiple infection with one or more HPV from this group which contain 26/51/82/69/34/73 types
in two (8%) patients were with types 16, 18, group 26/34 and type 53. HPV 16, 18 and 35 are the most common types of HPV in squamous cell carcinoma of the head and neck, and have a longer survival after treatment than HPV negative OPSCC\textsuperscript{24–26}. The reason for the rise in the incidence of HPV-positive OPSCC was in due course assigned to changes in sexual habits, since a relation between HPV-positive OPSCC, early sex debut and numbers of oral or vaginal patients was observed\textsuperscript{27}. The availability of prophylactic vaccines against HPV which has reduced the number of cervical cancers should also reduce the number of patients with tumours of the oral cavity, oropharynx and larynx, and should be looked into in the future. Although a small number of samples were included in the study, the research results point to the need for public health interventions in terms of better education of the public about the HPV virus and its role in this cause of cancer, as well as the importance of HPV vaccination in younger age groups (both sexes equally) as important preventive activities that can help prevent cancer.

This significant percentage of positive patients with human papilloma virus leads us to believe that human papilloma virus is an important risk factor for squamous cell carcinoma of the larynx, OPSCC, as well as due to the very prognosis as well as to the manner of oncology treatment and the introduction of preventive vaccine\textsuperscript{24–26, 28, 29}. The main limitation of the study is the time elapsed from the research to the analysis of the results. Although it was planned to conduct another study focusing on these cancer cells, COVID-19 pandemic has unfortunately stopped all other activities for the last three years, except for the fight against the pandemic. The goal of future research in Croatia is to compare and emphasize the results of our study that HPV plays an important role as a prognostic factor in squamous cell carcinomas of the larynx for its ability to block IGF-IR expression\textsuperscript{29}.

Conflict of Interest

None declared.

REFERENCES


