

# Prognostic Significance of HPV16 Infection in Oral Squamous Cell Carcinoma

## SUMMARY

**Background/Aim:** Many studies confirmed strong association between oncogenic genotypes of Human Papillomaviruses (HPVs) and some oropharyngeal and oral squamous cell carcinomas (OSCCs). The aim of this study was to determine the prognostic significance of HPV16 infection in OSCC in population of Montenegro i.e. correlation between HPV16 infection and disease free interval (DFI), disease recurrence and metastasis occurrence. **Material and Methods:** We examined surgically obtained specimens from 60 patients with OSCC (localized on the lower lip, tongue or/and floor of the mouth) in six years period. Tumor tissue samples were used for histological analysis and HPV16 genome PCR detection (primers for detection of E6 gene). Patients were followed up for the 3 years. **Results:** HPV16 infection was more commonly detected in females with OSCC ( $p=0.038$ ), while no correlation between this infection and disease recurrence ( $p=0.122$ ) and/or metastasis occurrence ( $p=0.934$ ) was found. DFI was not significantly different between patients with or without HPV16 infection ( $p=0.427$ ), but DFI was significantly shorter ( $p=0.003$ ) in females with oral HPV16 infection compared to male patients with this infection. **Conclusions:** Counting that HPV16 infection has a negative prognostic significance in OSCC, the HPV immunization program against genital HPV infection, would also have a positive impact on the frequency of HPV-mediated OSCC in Montenegrin population.

**Keywords:** HPV16-related Oral Cancer, Prognosis, Immunization

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## Introduction

Head and neck squamous cell carcinoma (HNSCC) is a significant public health problem, being between six most commonly diagnosed malignant tumors worldwide, with higher prevalence in developing countries<sup>1,2</sup>. Most important risk factors related with HNSCC occurrence are smoking and alcohol consumption, although in about a quarter of cases no connection with these traditional risk factors was found<sup>3,4</sup>.

Many studies confirmed connection between underlying infection with oncogenic genotypes of Human Papillomaviruses (HPVs) and a subset of HNSCC, particularly oropharyngeal and most likely a small subset of oral cancers<sup>5</sup>.

HPV is a small, DNA virus. Depending on the oncogenic potential HPVs are further classified into low-risk (LR-HPV) and high-risk (HR-HPV) subgroups by their genotype. LR-HPV infection is the cause of the skin, anal and genital warts, while high-risk genotypes are connected with head and neck, anal and genital cancers<sup>6-8</sup>. So far at least fifteen HR-HPV genotypes - 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 66, 68, 73 and 82 are proved to have oncogenic potential<sup>9</sup>.

The HPV involvement in oral and oropharyngeal carcinogenesis was first proposed by Syrjanen *et al.* who indicated the etiological role of HPV infection in the pathogenesis of head and neck cancers<sup>10</sup>. This was also further noticed in several studies<sup>11-13</sup> by observing histological similarities between oropharyngeal and

genital mucosa and isolating HR-HPV in oral squamous cell carcinoma (OSCC) tissue.

HPV is very common sexually transmitted infection. It is estimated that both genders get infected at least once during their life, but infection itself does not necessary cause the disease<sup>14</sup>. Similar to the cervical cancer, the most commonly isolated genotype in head and neck cancers is the genotype 16, while other HR-HPVs were almost never detected in this region<sup>15-17</sup>.

There are different opinions on the significance of HPV infection in head and neck cancer prognosis, depending on cancer location, geographic location and study sample size. Some authors<sup>18</sup> proposed that advanced OSCC patients with a solitary HPV16 infection have three times bigger chance for metastatic disease and more likely to live, according to significantly shorter DSS (disease specific survival) and OS (overall survival) values, but not DFS (disease free survival) values compared to HPV16- patients. According to these results, a solitary HPV16 infection is a significant risk factor for the distant metastases occurrence in a five-year period<sup>18</sup>. Contrary to these results, other authors<sup>19-21</sup> showed a positive impact of HPV infection on DSS and OS values.

Therefore, in this study we aimed to determine the prognostic significance of HPV16 infection in OSCC in population of Montenegro i.e. correlation between HPV16 infection and disease free interval (DFI), disease recurrence and metastasis occurrence.

## Material and Methods

The study was conducted at the Clinical Centre of Montenegro between 2012-2018 and included 60 patients with the indication for the surgical treatment of OSCC localized on the lower lip, tongue or/and floor of the mouth. The written informed consent was obtained from each patient and investigation was performed according the Helsinki Declaration principles (2002 version).

For histological analysis and HPV16 genome detection formalin-fixed and paraffin-embedded (FFPE) tumor tissue specimens collected during the surgery were used. Patients were followed up for three years. For measuring the time of survival, the beginning of treatment (date of primary surgery) until the time of disease recurrence (DFI-disease free interval) were encountered.

HPV16 DNA extraction from selected FFPEs, as well as from buccal swabs used as a control, was conducted using a KAPA Express Extract Kit (Kapa Biosystems, Inc., Wilmington, MA, USA), according to the providers' manual. Standard PCR method, using primers for HPV 16- E6 gene detection was performed and DNA concentration were measured spectrophotometrically.

Histological analysis was performed on standard haematoxylin/eosin sections by two

independent pathology specialists who had no insights in participants clinical and HPV16 status. Data on patients' gender, disease recurrence, metastasis occurrence and disease free interval (DFI) were correlated to HPV16 status.

For statistical analyses SPSS v.23.0 software was used. All data were categorical. Descriptive data were expressed as a percentage of a group for discrete measures. The Pearson's chi-squared test was used to analyse all the data and the level of significance was 0.05. Kaplan-Meier curves and the log-rank test were used for survival analysis.

## Results

This study enrolled 13 female and 47 male patients, all of the same ethnic background. The average patients' age was 62 (from 37-86 years). No correlation between oral HPV16 infection and disease recurrence ( $p= 0.122$ ) and/or metastasis occurrence ( $p= 0.934$ ) was found. HPV16 infection was significantly more common in females with OSCC ( $p= 0.038$ ). The association of mentioned clinico-pathological features and HPV16 are summarized in Table 1.

Table 1. Association of clinico-pathological features and HPV16 infection in OSCC

Category	Variable	Patients (n)	HPV16 + (%)	P-value
Gender	Female	13	6 (46.2)	0.038
	Male	47	8 (17)	
Disease Recurrence	+	9	4 (44.4)	0.122
Node Metastasis	+	15	4 (26.6)	0.934
Disease free interval (DFI)	+	60	14 (23.3)	0.427

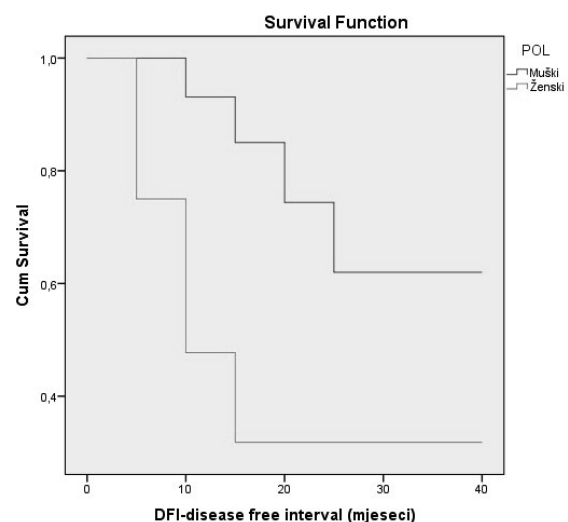


Figure 1. Disease free interval (DFI) between HPV16+ females ( $n = 6$ ) and HPV16+ males ( $n = 8$ ) was significantly different ( $p = 0.003$ )

There was no difference of DFI between HPV16-positive and HPV16-negative patients with OSCC (Log Rank test,  $p=0.427$ ). However, we detected a gender-based difference in DFI and found that DFI was statistically significant shorter (Log Rank test,  $p=0.003$ ) in females with oral HPV16 infection compared to male patients (Figure 1).

## Discussion

HPV infection rates in OSCCs varying from 0% to 100% according to different tumors studied<sup>22,23</sup>, dominantly in cancers of palatine tonsils and the base of the tongue<sup>24</sup>. These differences are partly due to the cancer location, geographic location and study sample size. It is well known that HPV infection is more prevalent in North America than in Europe and Asia, confirmed by the meta-analysis by Kreimer *et al.*<sup>16</sup>, which combines data from 60 studies from different geographical locations. In our study on 60 cases of OSCC, the HPV16 infection rate was 23.3%.

HR-HPV prevalence is about 26% in OSCC biopsies<sup>25</sup>. HPV16 genotype is the most prevalent HR-HPV in HPV-positive oral and oropharyngeal squamous cell carcinomas<sup>16</sup> with the rates of 75% in OSCC and 90% in oropharyngeal squamous cell carcinoma<sup>5</sup>.

In the study of the Serbian population, on a sample of 50 cancers of the tongue and floor of the mouth, the HPV infection was found in 64% of patients<sup>26</sup>, and analysis of HPV typing showed the following distribution: HPV16 - 31.2%, HPV18 - 18.7%, and HPV31 - 18.7%.

The study conducted on 50 Brazilian patients with OSCC (excluding oropharyngeal cancer), showed similar results to ours, with 24% HR-HPV positivity, mostly HPV16/18<sup>27</sup>, while in a Mexican population, the prevalence of HR-HPV was 43.5%<sup>28</sup>. These variations in prevalence may be the consequence of the different sample locations (oral cavity, oropharynx, hypopharynx and larynx), or sample numbers, differences in the sampling techniques, in the ethnic background of the subjects examined and in the applied detection methods (quantitative real-time PCR or in situ hybridization assays).

The frequency of oral HR-HPV infection (the most common type is HPV16) in healthy individuals with intact mucosa is very low (1.3%)<sup>29</sup>. Considering the fact that the HPV16 infection is the sexually transmitted anal-genital disease, the question remains why oral HPV infection is rare, especially considering the similarities of the oral and anal-genital epithelium? As a possible explanation, in addition to the different technical conditions for taking the samples, authors state that the oral mucosa may be more resistant to HPV infection<sup>30</sup>.

The results of our investigation show that 46.2% of women with OSCC were HPV16 positive, and only 17% of men with OSCC. This result indicates a statistically significant association between the presence of HPV16 and the gender of the patient. This gender distribution of HPV16 infection can be explained by the influence of the environmental factors, lifestyle and behavioral habits (sexual risk behavior).

HPV infection of the uterine cervix, as well as HPV infection of the mouth and oropharynx is in correlation with the risky sexual behaviour (oro-genital sex), and HPV16 genotype is dominantly present in many oral and oropharyngeal SCCs<sup>5</sup>. However, it is unclear whether HPV infection can be transmitted to the mouth in some other ways. According to that, one study confirmed that oral HPV infection in mothers was in correlation with the risk of oral HPV infection in their infants, and supported non-sexual HPV transmission possibility<sup>31</sup>. In addition, partners of women with cervical cancer generally have a higher incidence of tonsillar cancer, supporting the possibility of HPV transmission from the cervix to the oral cavity/oropharynx<sup>32</sup>.

DFI was significantly shorter in female patients with oral HPV16 infection compared to male patients with oral HPV16 infection. In many studies, HPV positive OSCC shows a better prognosis and reduced risk of disease recurrence compared with HPV negative tumors<sup>5,23</sup>. It seems that this is due to the ability of HPV positive cancer cells to induce apoptosis in response to DNA damage<sup>33</sup>.

While some authors confirmed that HPV16 infection is correlated with poor survival in patients with the advanced OSCC<sup>18</sup>, others showed that HPV16 positive patients had lower recurrence rates compared to HPV16 negative ones<sup>34</sup>. In none of these studies the calculated risk of recurrence and prognosis were related to the gender.

A prophylactic HPV vaccine is a potential tool for eradicating HPV-related diseases: a bivalent HPV vaccine (Cervarix, GlaxoSmithKline); a quadrivalent HPV vaccine (Gardasil, Merck); and a nonavalent HPV (9vHPV) vaccine (Gardasil9, Merck)<sup>35,36</sup>. Gardasil9 has been approved in Montenegro since 2019 ([www.cinmed.me](http://www.cinmed.me)), but the HPV vaccination program has been started in September 2022.

It is suggested that HPV prophylactic immunization should be primary directed to young girls, since the calculated risk of HPV-related malignancy in women is ten times bigger than in men. Due to the significant number of HPV positive penile, anal and head and neck cancers, in some countries HPV prophylactic vaccination is conducted in both boys and girls<sup>37</sup>. Many researches show high efficiency of vaccines in preventing HPV-related diseases, especially before HPV exposure, pointing out the importance of vaccination before the onset the sexual activity<sup>38,39</sup>.

## Conclusions

According to the results of this study which showed high prevalence of HPV16 infection and significantly shorter DFI in females with OSCC, we believe that the introduction of HPV prophylactic vaccine against genital HPV infection and cervical cancer, will have a positive impact on the frequency of HPV-related OSCCs and survival disease rate in our population.

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