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RESEARCH ARTICLE

Prevalence and clinical implications of the HPV16 infection in oral cancer in Montenegro – Evidence to support the immunization program

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ABSTRACT

Oral squamous cell carcinoma (OSCC) makes 85–95% of all malignances in the oral cavity. Increasing evidence shows that the Human Papillomaviruses (HPVs) are preferentially associated with some oropharyngeal and OSCCs, namely the genotype 16. The aim of the present study was to determine the prevalence and clinical implications of HPV16 infection in oral squamous cell carcinoma in population of Montenegro.

This study included 60 patients with OSCC (localized on the lower lip, tongue or/and floor of the mouth), surgically treated at the Clinical Centre of Montenegro from 2012 to 2018. Surgically obtained formalin-fixed and paraffin-embedded specimens were used for histopathological analysis and HPV16 genome detection using standard Polymerase Chain Reaction (primers for detection of E6 gene). Each individual was further followed up for the period of three years and for different clinico-pathological characteristics, including disease free interval (DFI).

The prevalence of HPV16 infection in OSCCs was 23.3% and the infection was significantly more common in female patients ($P = 0.038$). No significant correlation was detectable between HPV16 infection and the patients' age ($P = 0.302$), tumor site ($P = 0.125$), tumor grade ($P = 0.363$) and disease stage ($P = 0.995$). Observing the total sample the DFI was not significantly different for HPV16-positive versus HPV16-negative patients ($P = 0.427$), but a gender-based difference in DFI was observed, with the significantly shorter DFI (Log Rank test, $P = 0.003$) in HPV16 positive female patients compared to male patients ($P = 0.003$).

The results obtained in this study provide scientific evidence for the development of national HPV vaccination program in Montenegro.

KEYWORDS

Human Papilloma Virus, HPV16 infection, oral cancer, HPV vaccine

INTRODUCTION

Oral squamous cell carcinoma (OSCC) makes 85–95% of all malignances in the oral cavity [1]. With approximately 500,000 new cases each year, OSCC is the sixth most commonly diagnosed malignancy worldwide and the third most common cancer in developing countries [2].

It is well known that several factors are involved in oral carcinogenesis, including age, gender, ethnicity and lifestyle choices such as alcohol and tobacco consumption [3]. However, 15–20% patients with head and neck carcinomas have no history of tobacco or alcohol exposure [4]. Thus, other agents such as Human Papillomaviruses (HPVs) are being investigated as an etiological risk factor in oral carcinogenesis.

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The involvement of HPV infection in oral and oropharyngeal carcinogenesis was first proposed in 1983 by Syrjanen et al. [5] on the basis of several evidences: 1) strongly established etiological role of High Risk HPV (HR-HPV) in cervical squamous cell carcinoma; 2) morphological similarities between oropharyngeal and genital epithelia; 3) well-assessed broad epithelial-tropism of HPV and 4) detection of HR-HPV genotypes in samples of oral squamous cell carcinoma.

HPV is the most commonly sexually transmitted infection in the world. Sexually active women and men will be infected at least once in their lifetime, without necessarily developing any pathological disorders [6]. HPV is a small, double-stranded DNA virus classified into two categories: low-risk HPVs (LR-HPVs) responsible for anogenital and cutaneous warts, and high-risk HPVs (HR-HPVs) responsible for oropharyngeal (oral, tonsil, and throat areas) cancers and anogenital cancers, including cervical, anal, vulvar, vaginal, and penile cancers [7–9]. The International Agency for Research on Cancer (IARC) has identified 15 HR-HPV genotypes - 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 66, 68, 73 and 82 [10].

Although there are geographical differences in HPV genotype prevalence and distribution, genotype 16 is the most commonly detected in cervical cancer biopsies (55%), as well as in head and neck carcinomas (85–95%) worldwide [11]. Other oncogenic HPV genotypes detected in invasive cervical cancer (HPV18, 31, 33, 35, 45, 56, 58 and 59) are rarely or never detected in head and neck carcinoma biopsies [12, 13].

A prophylactic HPV vaccine is a potential tool for eradicating HPV-related diseases. There are currently three vaccines approved by the United States Food and Drug Administration: a bivalent HPV vaccine containing virus-like particles (VLPs) of the HR-HPV 16 and 18 genotypes (Cervarix, GlaxoSmithKline); a quadrivalent HPV vaccine that includes VLPs of HPV 16 and 18 along with the 2 low-risk (LR) genotypes HPV 6 and 11 (Gardasil, Merck); and a nonavalent HPV (9vHPV) vaccine containing VLPs of 7 HR genotypes-namely, HPV 16, 18, 31, 33, 45, 52, and 58 - plus HPV 6 and 11 (Gardasil9, Merck) [14, 15]. Although Gardasil9 has been approved in Montenegro since 2019 (www.cinmed.me), the HPV vaccination program has yet to be implemented.

Epidemiological surveillance of HPV infection and related diseases represents a crucial topic for monitoring and evaluation of the three currently available antiviral prophylactic vaccines, as well as their acceptance all over the world [16].

Montenegro is a country with small population and only reliable data existing are on HPV infection prevalence in women with suspected cervical dysplasia [17]. The investigation on prevalence and clinical implications of oral HPV infection in our population has never been done and we believe that the results obtained in this study would be valuable for creating future national strategies for eradication of HPV - related malignancies in Montenegro.

Therefore, the aim of the present study was to determine the prevalence and clinical implications of HPV16 infection in OSCC in population of Montenegro.

MATERIALS AND METHODS

The study was conducted at the Clinical Centre of Montenegro from 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 study was performed according to the ethical principles governing medical research and human subjects as laid down in the Helsinki Declaration (2002 version). All participants were informed of the procedures and furnished written informed consent.

Surgically obtained formalin-fixed and paraffin-embedded (FFPE) specimens were used for HPV16 genome detection and histopathological analysis and each individual was further followed up for the period of three years. The duration of survival was measured from the beginning of treatment (date of primary surgery) until the time of disease recurrence (DFI-disease free interval).

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), as recommended by the manufacturer. Standard Polymerase Chain Reaction (PCR method) using primers for detection of HPV 16 - E6 gene was performed, and DNA concentration was measured spectrophotometrically.

Histopathological analysis was performed on standard HE sections by two pathologists without prior knowledge of patients' clinical data and HPV16 status.

Data on patients' gender, age, tumor site, histological grade, disease stage, disease recurrence (disease free interval - DFI) were correlated with HPV16 status.

As no reliable information on tobacco and alcohol consumption was available, such data were not included in the study.

For statistical analyses, SPSS v.23.0 software (SPSS Inc., Chicago, IL, USA) 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. Survival analysis was performed using Kaplan-Meier curves and the log-rank test.

RESULTS

In our cohort, there were 13 female and 47 male patients and all of the same ethnic background. The youngest patient was 37 and the oldest was 86, the average age was 62.

The HPV16 infection was detected in 14 of 60 cases, thus the prevalence of HPV16 infection in OSCC was 23.3% (Table 1).

Table 1. Prevalence of HPV16 infection in OSCC

Presence of HPV16 Infection	N (%)	*p*
Yes	14 (23.3%)	$P < 0.001$
No	46 (76.7%)	

No significant correlation was detectable between HPV16 infection and the patients' age and HPV16 positive tumors were not associated with any age group ($r = -0.135$, $P = 0.302$). No correlation was found between oral HPV16 infection and: tumor site ($r = -0.200$, $P = 0.125$), tumor grade ($r = -0.120$, $P = 0.363$), disease stage ($r = -0.001$, $P = 0.995$), disease recurrence ($r = 0.202$, $P = 0.122$) and/or metastasis occurrence ($r = -0.011$, $P = 0.934$). Data on correlation between HPV16 infection and mentioned clinico-pathological characteristics are summarized in Table 2.

Significant correlation was found between oral HPV16 infection and patients' gender. HPV16 infection was significantly more common in female patients with OSCC ($r = -0.284$, $P = 0.038$) (Fig. 1).

Observing the total sample, the DFI was not significantly different between HPV16-positive versus HPV16-negative patients with OSCC (Log Rank test, $P = 0.427$) (Fig. 2). However, we observed a significant gender-based difference in DFI and found that DFI was significantly shorter (Log Rank test, $P = 0.003$) in female patients with oral HPV16 infection compared to male patients with oral HPV16 infection (Fig. 3).

Table 2. Statistical association of clinical factors and HPV16 status

Category	Variable	Patients (*n*)	HPV16 + (%)	*P*-value
Age	≤40	2	0 (0)	0.302
	>40 and <60	25	5 (8.3)	
	≥60	33	9 (15)	
Sex	Female	13	6 (46.2)	0.038
	Male	47	8 (17)	
Stage	I	23	3 (13)	0.995
	II	13	4 (30.7)	
	III	9	4 (44.4)	
	IV	15	3 (20)	
Tumor grade	Well	35	8 (23)	0.363
	Moderately	23	5 (21.7)	
	Poorly	2	1 (50)	
Tumor site	Lower lip	28	4 (14)	0.125
	Tongue	22	5 (22.7)	
	Floor of mouth and tongue	10	5 (50)	
Disease Recurrence	+	9	4 (44.4)	0.122
Metastasis (node)	+	15	4 (26.6)	0.934

Clinical categories and the variables were examined for significant associations with HPV16 status. Statistical significance was assessed by Fisher's exact and Pearsons's chi-square test ($P < 0.05$).

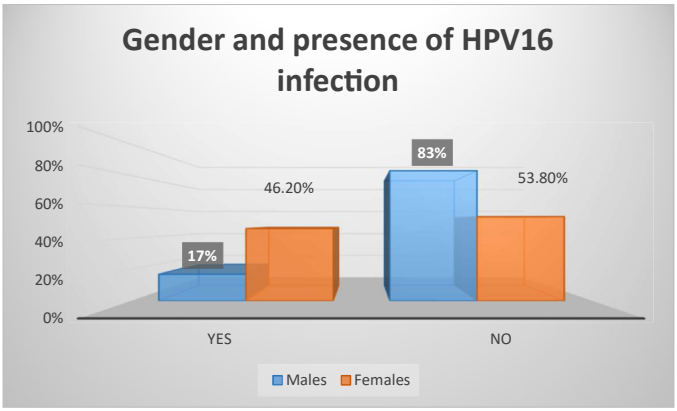

Fig. 1. Gender and presence of HPV16 infection in OSCC. HPV16 infection was significantly more common in female patients with OSCC ($P = 0.038$)

DISCUSSION

To our knowledge, this is the first study on HPV16 positive OSCC in Montenegro. The only available data on HR-HPV infection prevalence in Montenegrin population relies on the study conducted among women with suspected cervical dysplasia. In this research, high HR-HPV infection prevalence (40.6%) was shown among examined women, with the genotype 16 being the most prevalent in cases with proven dysplasia (28.9%) [17].

In our study on 60 cases of OSCC, we also found a significantly high prevalence of HPV16 infection (23.3%).

In the literature, the reported rates of detection of HR-HPV in head and neck squamous cell carcinomas range from 0 to 100% [18]. The study conducted on 50 Brazilian patients with OSCC (excluding oropharyngeal cancer) had the results similar to ours, with 24% HR-HPV positivity, mostly HPV16/18 [19], while in a Mexican patient cohort, a much higher frequency of HR-HPV positivity (43.5%) was found [20]. These variations in reported prevalence may be owing to lumping together of essentially different lesions (oral cavity, nose and para-nasal sinuses, naso-pharynx, oro-pharynx, hypo-pharynx and larynx), to small sample numbers, to differences in the sampling techniques, in the ethno-geographic origins of the subjects examined and in the HPV detection methods applied (quantitative real-time PCR or in situ hybridization assays) [21].

It is generally accepted that HPV DNA is detected in about 26% of biopsy specimens of head and neck squamous cell carcinomas [22]. HPV16 DNA was found to be the most prevalent HPV genotype in HPV-cytopositive oral and oropharyngeal squamous cell carcinoma [12, 22] and was detected in about 75% of cases of HPV-cytopositive OSCC and in about 90% of cases of HPV-cytopositive oropharyngeal squamous cell carcinoma [23, 24].

HPV infection of the mouth and of the oropharynx, like HPV infection of the uterine cervix, is associated with high-risk sexual behaviour (in particular with oro-genital sex), and high-risk HPV genotypes (in particular HPV16), are

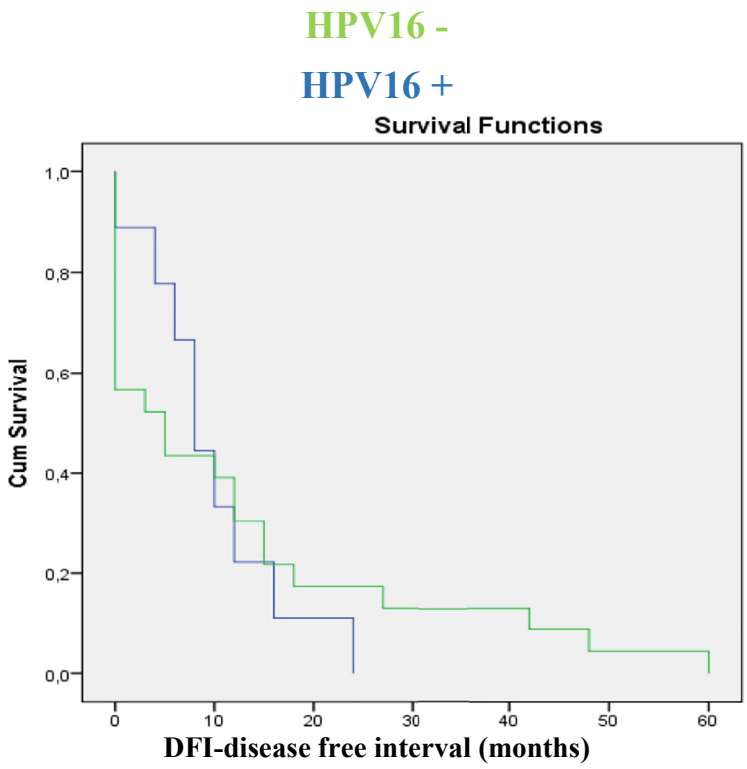

Fig. 2. Disease free interval (DFI) in patients with oral HPV16 infection ($n = 14$) and without oral HPV16 infection ($n = 46$). There was no significant difference among the examined groups ($P = 0.427$)

present in many oral and oropharyngeal SCC where in some cases they probably play an essential aetiological role [23]. Sexual behaviour has consistently been associated with increased oral HPV prevalence, supporting the sexual transmission of the virus.

However, it is unclear whether HPV can be casually transmitted (i.e. transmitted non-sexually) to the oral cavity. According to that, one family study found persistent oral HPV infection in mothers was associated with increased risk of persistent oral HPV infection in their infants, suggesting that non-sexual transmission might occur [25]. In addition, partners of women with cervical cancer also have a higher incidence of tonsillar cancer than the general population, supporting the possibility of transmission from the cervix of an infected women to the oral cavity/oropharynx during oral sex [26].

Although in general, the OSCC is more commonly diagnosed in men, interestingly in our cohort, where we also had more male patients examined, HPV16 infection was significantly more prevalent in females. Moreover, among our study group DFI was significantly shorter in female patients with oral HPV16 infection compared to male patients with oral HPV16 infection.

While some studies confirmed that HPV-16 infection enhanced the risk of distant metastasis and poor survival in patients with advanced OSCC [27], another studies showed that cases positive for HPV-16 had lower recurrence rates compared with that for their negative counterparts, indicating an association between HPV-16 infection and a good prognosis in OSCC [28]. In none of these studies the calculated risk of recurrence and prognosis were in relation to the gender.

The three types of currently approved HPV vaccines, including bivalent, tetravalent, and nonvalent vaccines, are effective in reducing HPV infection rate and HPV-related disease incidence, as reported in several world regions. This effectiveness lies in the fact that they target and induce immunity against LR- and HR-HPVs responsible for 70 and 90% of genital and cutaneous warts and cancers, respectively. 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 [29, 30].

The World Health Organization suggests that HPV prophylactic vaccination should be primary directed to young girls, since the calculated risk of HPV-related malignancy in women is ten times bigger than in men. It is estimated that through high vaccine coverage of girls, the heard immunity would be established among heterosexual men [31, 32].

On the other hand, following this logic, homosexual men would stay unprotected and also in many countries the percentage of vaccinated girls is still low. Taking into account all of these facts and the fact that significant number of penile, anal and head and neck cancers are in relation to HPV infection in some countries, HPV prophylactic vaccination is conducted in both boys and girls [33–36].

Results of the previously mentioned research in Montenegro are indicating that prophylactic nonavalent vaccine can potentially prevent approximately 90% of HR-

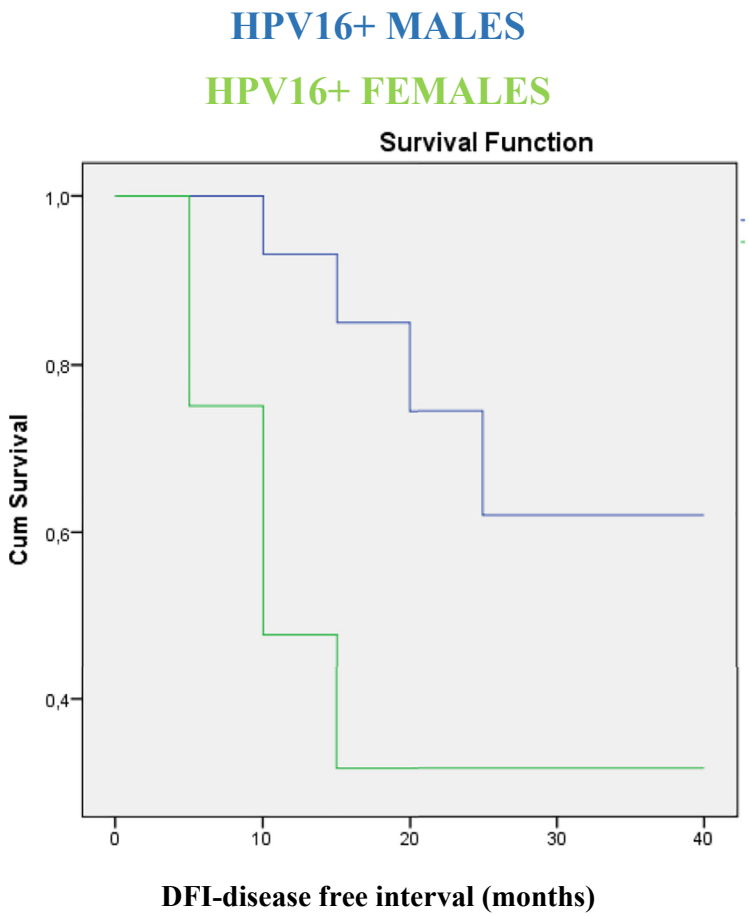

Fig. 3. Disease free interval (DFI) in HPV16+ female patients ($n = 6$) compared to HPV16+ male patients ($n = 8$). There was significant difference among the examined groups ($P = 0.003$)

HPV infections and 60% of cervical dysplasia cases in Montenegrin women [17].

Taking into account that finding, along with the results of the current study, we believe that the introduction of HPV vaccination as a public health measure against genital HPV infection and cervical cancer will most probably have a favourable impact on the frequency of HPV-mediated oral squamous cell carcinoma. The results obtained in this study provides important scientific evidence for the development and implementation of the national HPV vaccination program in Montenegro.

Moreover, our results which show higher prevalence of HPV16 infection and shorter DFI in women with OSCC, speak in favor of HPV vaccination, primarily directed to girls, as being beneficial in our population.

Conflict of interest: The authors have no conflict of interest to declare.

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