INFEKTOL GLASN 2022;42(2):51-56
https://doi.org/10.37797/ig.42.2.2

Professional paper | Stručni rad

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

Ivica Nekić[1], Alan Medić[2,3], Dinko Puntarić[4], Tatjana Nemeth Blažić[5], Nataša Lisica Šikić[6], Suzana Konjevoda[3,7], Diana Nonković[8], Jadranka Sambunjak[9], Boris Dželalija[3]

[1] Department of Otorhinolaryngology, Zadar General Hospital, 23000 Zadar, Croatia
[2] Department of Epidemiology, Zadar Institute of Public Health, 23000 Zadar, Croatia
[3] Department of Health Studies, University of Zadar, 23000 Zadar, Croatia
[4] Croatian Catholic University, 10000 Zagreb, Croatia
[5] Croatian Institute of Public Health, Division for Epidemiology of Communicable Diseases, Reference Centre of the Epidemiology of the Ministry of Health, 10000 Zagreb, Croatia
[6] Department of Pathology, Cytology, and Forensic Medicine, Zadar General Hospital, 23000 Zadar, Croatia
[7] Department of Ophthalmology, Zadar General Hospital, 23000 Zadar, Croatia
[8] Department of Epidemiology, Teaching Institute of Public Health, Split and Dalmatia County, 21000 Split, Croatia
[9] Department of Laboratory Medicine, Zadar General Hospital, 23000 Zadar, Croatia

Keywords:
carcinomas
larynx
oropharynx
oral cavity
HPV
epidemiology
Zadar
Croatia

Ključne riječi:
karcinomi
grkljan
orofarinks
usna šupljina
HPV
epidemiologija
Zadar
Hrvatska

Primljeno: 09-01-2022
Received: 09-01-2022

Prihvaćeno: 30-01-2022
Accepted: 30-01-2022

✉ **Corresponding author:**
Associate Professor Alan Medić, MD, PhD,
Zadar Institute of Public Health,
Ljudevita Posavskog 7, HR 23000 Zadar, Croatia;
E-mail: alan.medic@zjz.htnet.hr

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 specified 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[2]. 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[8]. More than 70% of OPSCC cases and over 50% of tonsillar cancers in the USA have been associated with high-risk HPV types[9]. 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[6-9]. 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, 16]. 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 high 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, 15]. 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, 18]. 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 Gane 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

HPV 16, 18, 53 and detection group 26/34 (contain HPV types 26/51/82/69/34/73) by the FAM fluorescence growth.

For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for GAPDH gene whose amplification is indicated in the HEX fluorophore fluorescence channel.

HPV were separated by this method into four groups: KIT containing MasterMix HPV 16, KIT containing MasterMix HPV 18, KIT containing MasterMix HPV 53 and KIT containing MasterMix HPV 26/34.

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).

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

Tablica 1. Broj pacijenata liječenih od raka glave i vrata u Općoj bolnici Zadar u razdoblju od deset godina prema spolu i lokalizaciji karcinoma

Cancer location	Male (%)	Female (%)	Total (%)
Larynx	56 (90.3)	6 (9.7)	62 (62.6)
Oral cavity	22 (84.6)	4 (15.4)	26 (26.3)
Oropharynx	11 (100)	0	11 (11.1)
All locations	89 (89.9)	10 (10.1)	99 (100)

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).

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

Tablica 2. HPV status uzoraka tkiva pacijenata liječenih od karcinoma glave i vrata u Općoj bolnici Zadar u razdoblju od deset godina

Cancer location	HPV+ (%)	HPV - (%)
Larynx (n=62)	16 (25.8)	46 (74.2)
Men	14 (87.5)	
Women	2 (12.5)	
Oral cavity (n=26)	7 (26.9)	19 (73.1)
Men	6 (85.7)	
Women	1 (14.3)	
Oropharynx (n=11)	2 (18.2)	9 (81.8)
Men	2 (100)	
Women	0	
All locations (n=99)	25 (25.3)	74 (74.7)
Men	22 (88.0)	
Women	3 (12.0)	

Table 3. HPV type detected in carcinoma tissue samples of patients operated due to head and neck cancers in Zadar General Hospital in the period of ten years by cancer location

Tablica 3. Tip HPV-a otkriven u uzorcima tkiva karcinoma bolesnika operiranih od karcinoma glave i vrata u Općoj bolnici Zadar u razdoblju od deset godina prema lokalizaciji karcinoma

	HPV types			Total (%)
	16	16,53, group 26/34*	16,18, 53 group 26/34*	
Larynx	10 (62.5%)	5 (31.2%)	1 (6.3%)	16 (100%)
Oral cavity	3 (42.9%)	3 (42.9%)	1 (14.2%)	7 (100%)
Oropharynx	2 (100%)	0 (0%)	0 (0%)	2 (100%)
All locations	15 (60%)	8 (32%)	2 (8%)	25 (100%)

*Group 26/34 mean: Multiple infection with one or more HPV from this group which contain 26/51/82/69/34/73 types

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 type53 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

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[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 partners was observed[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[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[29].

Conflict of Interest

None declared.

REFERENCES

[1] Gatta G, Botta L, Sanchez MJ, Anderson LA. Prognosis and improvement for head and neck cancers diagnosed in Europe in early 2000's. Eur J Cancer 2015;51:2130.

[2] RARECAREnet [Internet]. [cited 2015 Feb 15]. Available from: http://www.rarecarenet.eu/rarecarenet/images/indicators/Incidence.pdf.

[3] Lubin JH, Purdue M, Kelsey K, et al. Total exposure and exposure rate effects for alcohol and smoking and risk of head and neck cancer: a pooled analysis of case-control studies. Am J Epidemiol 2009;170:937–47.

[4] Mork J, Lie AK, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125–31.

[5] Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 2006;15:1765–77.

[6] World Cancer Research Fund. American Institute for Cancer Research. Food, Nutrition and Physical activity, and Prevention of Cancer: a Global Perspective. Washington DC: AICR; 2007.

[7] Eriksen M, Mackay J, Ross H. The tobacco atlas. 4th ed. Atlanta: American Cancer Society; 2012.

[8] Aupérin A. Epidemiology of head and neck cancers: an update. Curr Opin Oncol 2020 32:178-186. doi: 10.1097/CCO.0000000000000629.

[9] Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010;11:781-9. doi: 10.1016/S1470-2045 (10) 70017-6.

[10] Qendri V, Bogaards JA, Baussano I, Lazzarato F, Vänskä S, Berkhof J. The cost-effectiveness profile of sex-neutral HPV immunization in European tender-based settings: a model-based assessment. Lancet Public Health 2020;5:e592-e603. doi: 10.1016/S2468-2667(20)30209-7.

[11] D'Souza G, Wentz A, Kluz N, et al. Sex Differences in Risk Factors and Natural History of Oral Human Papillomavirus Infection. J Infect Dis 2016;213:1893-6. doi: 10.1093/infdis/jiw063.

[12] Chaturvedi AK, Graubard BI, Broutian T, et al. NHANES 2009-2012 Findings: Association of Sexual Behaviors with Higher Prevalence of Oral Oncogenic Human Papillomavirus Infections in U.S. Men. Cancer Res 2015;75:2468–2477. doi: 10.1158/0008-5472.CAN-14-2843.

[13] Schnelle C, Whiteman DC, Porceddu SV, Panizza BJ, Antonsson A. Past sexual behaviors and risks of oropharyngeal squamous cell carcinoma: A case-case comparison. Int J Cancer 2017;140:1027–1034. doi: 10.1002/ijc.30519.

[14] Ernani V, Saba NF. Oral Cavity Cancer: Risk Factors, Pathology, and Management. Oncology 2015;89:187-95.

[15] Lore&Medina. Atlas of Head and Neck Surgery. 4th ed. Amsterdam: Elsevier; 2009.

[16] Bruni L, Diaz M, Barrionuevo-Rosas L, et al. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Glob Health 2016;4:e453-63. doi: 10.1016/S2214-109X(16)30099-7..

[17] Lind PO, Syrjanen SM, Syrjanen KJ, Koppang HS, Aas E. Local immunoreactivity and human papillomavirus (HPV) in oral precancer and cancer lesions. Scand J Dent Res 1986;94:419-26.

[18] Tumban E. A Current Update on Human Papillomavirus-Associated Head and Neck Cancers. Viruses 2019;11:922. doi: 10.3390/v11100922.

[19] Näsman A, Du J, Dalianis T. A global epidemic increase of an HPV-induced tonsil and tongue base cancer - potential benefit from a pan-gender use of HPV vaccine. J Intern Med 2020;287:134-152. doi: 10.1111/joim.13010.

[20] Devaraja K, Aggarwal S, Verma SS, Gupta SC. Clinico-pathological peculiarities of human papilloma virus driven head and neck squamous cell carcinoma: A comprehensive update. Life Sci 2020;245:117383. doi: 10.1016/j.lfs.2020.117383.

[21] Rooper LM, Windon MJ, Hernandez T, et al. HPV-positive Squamous Cell Carcinoma of the Larynx, Oral Cavity, and Hypopharynx: Clinicopathologic Characterization With Recognition of a Novel Warty Variant. Am J Surg Pathol 2020;44:691-702. doi: 10.1097/PAS.0000000000001433.

[22] Kaplon AW, Galloway TJ, Bhayani MK, Liu JC. Effect of HPV Status on Survival of Oropharynx Cancer with Distant Metastasis. Otolaryngol Head Neck Surg 2020;163:372-374. doi: 10.1177/0194599820913604.

[23] Dogantemur S, Ozdemir S, Uguz A, et al. Assessment of HPV 16, HPV 18, p16 expression in advanced stage laryngeal cancer patients and prognostic significance. Braz J Otorhinolaryngol 2020;86:351-357. doi: 10.1016/j.bjorl.2019.11.005.

[24] Janecka-Widła A, Mucha-Małecka A, Majchrzyk K, et al. Active HPV infection and its influence on survival in head and neck squamous-cell cancer. J Cancer Res Clin Oncol. 2020;146:1677-1692. doi: 10.1007/s00432-020-03218-6.

[25] Du E, Mazul AL, Farquhar D, Brennan P, et al. Long-term Survival in Head and Neck Cancer: Impact of Site, Stage, Smoking, and Human Papillomavirus Status. Laryngoscope 2019; 129:2506-2513. doi: 10.1002/lary.27807.

[26] Bukhari N, Joseph JP, Hussain SS, et al. Prevalence of Human Papilloma Virus Sub Genotypes following Head and Neck Squamous Cell Carcinomas in Asian Continent, A Systematic Review Article. Asian Pac J Cancer Prev 2019;20:3269-3277. doi: 10.31557/APJCP.2019.20.11.3269.

[27] D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009;199:1263-9. doi: 10.1086/597755.

[28] Gazzaz MJ, Jeffery C, O'Connell D, Harris J, Seikaly H, Biron V. Association of human papillomavirus related squamous cell carcinomas of the oropharynx and cervix. Papillomavirus Res 2019;8:100188. doi: 10.1016/j.pvr.2019.100188.

[29] Ben Elhadj M, Fourati A, El Amine O, et al. Prevalence and Prognostic Value of HPV among Tunisian Patients with Laryngeal Cancer and Relationship between DNA HPV and p16, IGF-1R, Survivin, p53 Expressions. Ann Otol Rhinol Laryngol 2020;129:863-871. doi: 10.1177/0003489420918280.