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SQUAMOUS CELL CARCINOMA OF THE LARYNX DUE TO HUMAN PAPILLOMAVIRUS INFECTION

Victória Gayoso Neves Soares Pereira

Helena Messias Gomes

Pedro Henrique Gunha

Ana Paula de Andrade

Livia Bittencourt Tahan

Sergio César Cordeiro Neto

Rafaela Schiessl

Gabriel Julião de Souza

Camilla Rodrigues do Couto Salturi

Maria Clara Messias Gomes

Eduardo Morais de Castro
PhD in Biotechnology Applied to Child and Adolescent Health Faculdades Pequeno Príncipe
Curitiba-PR

Paulo Eduardo Przysiezny
Master's Degree in Oral and Maxillofacial Surgery Faculdades Pequeno Príncipe
Curitiba-PR

INTRODUCTION

The Human Papillomavirus, known by the acronym HPV, is a diverse group of viruses belonging to the Papillomaviridae family. Its name derives from the tendency of these viruses to cause the abnormal growth of warts (papillomas) on the mucous membranes and skin, with a higher incidence in the genital areas. However, it can affect other regions, including the larynx (LURIA *et al.,* 2023). There are more than 100 types of HPV identified, a significant proportion of which have the potential to infect the anogenital and oropharyngeal regions. The location of the lesions varies according to the type of HPV and the site of primary contact. They can be divided into two groups: those at high risk of malignant neoplasia (mainly types 16 and 18) and those at low risk (mainly types 6 and 11) (RIVERA *et al.*, 2022).

How HPV is transmitted has not yet been fully elucidated. When it comes to laryngeal infections in children, there is a hypothesis that transmission occurs vertically, i.e. from mother to baby, during childbirth. In adults, transmission is believed to be related to unprotected oral sex (SOHELLI *et al.*, 2021).

The larynx is an organ located in the upper part of the respiratory system, with vital functions related to breathing, sound production (speech) and protection of the airways. Involvement of the larynx by HPV usually occurs due to infection by specific types of the virus. The main types that can settle in the oropharynx are HPV 6, 11 and 16 (RIVERA *et al.*, 2022).

The action of HPV is multifactorial, involving immune, genetic and environmental factors that determine the progression or regression of infections. HPV invades the epithelial cells of the skin and mucous membranes, where it uses cellular mechanisms to replicate itself. It is able to integrate itself into the host's DNA and interfere with cellular control processes, promoting uncontrolled cell multiplication. This can result in the development of warts (papillomas) or, in more serious cases, precursor lesions (dysplasia) or even malignant neoplasms (carcinoma) (LURIA *et al.,* 2023), and co-infection with other sexually transmitted infections (STIs) can increase the risk of cancer (MOSMANN *et al.*, 2019).

METHODOLOGY

The methodology used in this study was a review of technical books and an integrative review of scientific articles published at any time in the following databases: PubMed, Scielo, BVS and Google Scholar. The following Booleans were used: HPV; HPV *and* Larynx.

EPIDEMIOLOGY AND HISTORY IN BRAZIL

Human papillomavirus (HPV) infection represents a significant global health problem, with a considerable prevalence in various regions of the world. It is estimated that billions of people are infected with HPV at some point in their lives and its geographical distribution varies, with higher infection rates in low- and middle-income countries, where lack of access to medical care and sexual health education can contribute to the spread of the virus (DUARTE *et al.*, 2017).

HPV is a common virus that affects both men and women and is transmitted mainly through sexual intercourse, making it one of the most common STIs (SHAH *et al.*, 2017).

The global prevalence of HPV shows that the virus has high rates worldwide: the World Health Organization (WHO) estimated in 2018 an incidence of approximately 291 million women and 288 million men (SHAH *et al.*, 2017). However, it is important to note that the prevalence and incidence of HPV can vary between different regions and populations (SHAH *et al.*, 2017).

In Brazil, HPV rates are also a public health concern: the country is considered to have a high prevalence, with around 32.8% of women between the ages of 16 and 25 infected with the virus, according to data from the Ministry of Health in 2019. This represents a serious problem, since HPV is responsible for around 70% of cervical cancer cases in Brazil, according to INCA (INCA, 2023).

With this, some Brazilian epidemiological studies try to justify the high number of HPV complications: a study carried out in the Amazon region found an overall prevalence of HPV infection of 16.4% among women from riverside communities and, of this number, showed that the majority of women had never had a Pap smear test, which is an important screening tool for HPV-related cervical cancer (DUARTE *et al.*, 2017).

As for HPV-related laryngeal cancer, unlike cervical cancer, it is important to note that the majority of cases are not caused by HPV. However, it is estimated that HPV is responsible for around 30% of laryngeal cancer cases (SHAH *et al.*, 2017). In Brazil, although there are no specific figures available, the incidence of HPV-related laryngeal cancer is becoming more recognized and studied. However, to date, there have been few studies and investments in the area, which makes it impossible to have more concrete and accurate figures (DUARTE *et al.*, 2017). Some studies show that the age group most affected by laryngeal cancer is between 46 and 55 years old (BETZ, 2019; EGAWA *et al.*, 2015; MOSMANN *et al.*, 2019; VILLAGÓMEZ-ORTIZ *et al.*, 2016; TRZCINSKA *et al.*, 2019).

CLINICAL PICTURE

The HPV virus is often found to be an important cause of lesions in the oral and anogenital cavity, in both sexes, and often incubates for years. These lesions are characterized as exophytic, papillomatous, cauliflower-like lesions at the primary site, called condylomata acuminata (REIS *et al.*, 2020). The lesion can also occur in a sessile or pedunculated form, with an average size of 1.0 cm to 1.5 cm, the color varies according to the keratinization, being reddish, pink or whitish.

The population's lack of knowledge about where HPV affects, especially the oropharynx, is a risk factor for the development of cancer, with late diagnosis and follow-up. The genital presentation of HPV is still perceived by the population and often followed up in consultations and exams, but the oropharyngeal forms may not be recognized. In this sense, there is a lack of awareness about the forms of HPV, which may be aggravated by the fact that 44.9% of the population aged around 15 has not completed elementary school in Brazil (BURLAMAQUI, 2017).

ANOGENITAL LESIONS

Anogenital lesions can manifest in clinical, subclinical and latent forms. The clinical presentation is the appearance of warts, called condylomata acuminata, flat or papillomatous, single or multiple. The surface is matte and velvety. Most lesions are asymptomatic, but there may be complaints of itching, pain or bleeding. On the other hand, subclinical lesions are not visible macroscopically and have no symptoms. The latent form also does not manifest symptoms or the appearance of lesions, i.e. it is a "dormant" form of the disease (MOURA, 2019). Anogenital involvement can involve the vulva, vagina, perianal region, anus, penis, scrotum and pubic region (MS, 2023). In men, the most common region is the inner leaflet of the foreskin and, in women, the vagina, vulva and cervix. This is why regular preventive cytopathological examinations are so important (CARVALHO, 2021).

ORAL LESIONS

Among the oral regions, the tongue, lingual brake and soft palate are the most affected, however, there may be lesions on the uvula, lips and floor of the mouth (LIMA, 2020). In one study, the most affected regions were: larynx 42%, alveolar ridge and hard palate 4%, tongue 27%, floor of mouth 4%, lip 9% and tonsil 9% (BETZ, 2019; EGAWA *et al.,* 2015; MOSMANN *et al.*, 2019; VILLAGÓMEZ-ORTIZ *et al.*, 2016; TRZCINSKA *et al.*, 2019). The Oral lesions can be benign when they come from types 2, 4, 6, 11, 13 and 32, and malignant when they are associated with HPV types 16 and 18 (REIS *et al.*, 2020). The main target of the virus is the cells of the basal layer of the oral mucosa. As there is an inflammatory process often present in the gums, there is an increase in the division of epithelial cells in the tissue, which aids viral replication. In addition, the presence of periodontal bacteria in the oral mucosa can contribute to the persistence of HPV and the development of squamous cell carcinomas (SYRJÄNEN, 2018).

COMPLICATIONS

HPV infection of the larynx can give rise to a spectrum of complications, ranging from benign lesions to more serious conditions, with notable impairment of vocal function, breathing and quality of life for affected individuals (FERLAY *et al*., 2012). A thorough understanding of these complications is crucial to facilitate early diagnosis, proper management and the implementation of effective preventive strategies. Three main complications emerge when HPV affects the larynx: recurrent respiratory papillomatosis, dysplasia and squamous cell carcinoma of the larynx.

RECURRENT RESPIRATORY PAPILLOMATOSIS

The HPV virus, most commonly types 6 and 11, is the main etiological agent of recurrent respiratory papillomatosis (RRP), characterized by the development of benign tumors in the upper respiratory tract, called papillomas, in the vocal folds and adjacent areas of the larynx (MATOS, 2013). Papillomatosis can be acquired through vertical transmission during childbirth or through sexual contact (KAYODE, 2012).

These lesions can recur and develop throughout the respiratory tract, especially in areas of constriction, drying and oxygenation of the airways (MOUNTS and KASHIMA, 1984). In this sense, the junctions of the different epithelia, vocal folds, nasal vestibule and nasopharynx of the soft palate are the most frequent foci of the disease (KASHIMA *et al*., 1993), which progresses to a great extent, with the potential for the development of malignant neoplasia (MATOS, 2013; KASHIMA *et al*., 1993; DERKAY, 2001).

The symptoms of the disease can include hoarseness and stridor, indicative of upper airway obstruction, as well as other respiratory signs and symptoms such as coughing, wheezing, chronic dyspnea and choking. In more serious cases, it can lead to syncope due to complete airway obstruction (DERKAY and WIATRAK, 2008).

LARYNGEAL DYSPLASIA

Persistent HPV infection in the larynx can trigger dysplasia, with alterations in the cells of the squamous epithelium of the larynx. Laryngeal dysplasia is considered a precursor lesion to squamous cell carcinoma. Risk factors such as smoking and excessive alcohol consumption can increase the likelihood of dysplasia developing. Diagnosis is made by anatomopathological examination, and dysplasia can be graded as mild, moderate or severe (JOHNSON *et al*., 2021).

LARYNGEAL CANCER

In some cases, persistent HPV infection in the larynx can develop into an even more serious condition - squamous cell carcinoma (SCC). This neoplasm arises from the squamous epithelium lining the larynx.

The etiological relationship between laryngeal involvement by SCC and HPV infection, especially type 16, has been established (DOGANTEMUR, 2020). This relationship was strengthened in a study that found an association between invasive SCC and a strong and diffuse expression of the p16 protein by immunohistochemistry, indicating the presence of high-risk HPV (ZORZANELLI *et al.*, 2022).

Histopathological evaluation of the biopsied tissue is crucial for confirming the diagnosis, determining the stage of the disease and the most appropriate therapeutic approach. Early detection is essential for a better prognosis, since laryngeal SCC can metastasize and progress to advanced disease by compromising cervical lymph nodes, lungs and bones (BUEXM, 2018).

DIAGNOSIS

The history of the lesion and the patient's anamnesis are essential for suspecting HPV, as is its appearance. The clinical history should take into account that the virus can remain inactive for months to years after contact, and should be associated with a careful clinical examination (REIS *et al.*, 2020).

The gold standard for diagnosis and treatment of HPV lesions is excisional biopsy, with anatomopathological evaluation. In the case of papillomas, papillomatous hyperplasia of the squamous epithelium is observed, as well as the presence of koilocytes, cells with hyperchromatic, irregular nuclei and a perinuclear halo, suggestive of HPV infection (MARTINS *et al.*, 2013; QUREISHI *et al.*, 2017). For squamous cell carcinomas, there is epithelium with cellular atypia, dyskeratosis and, in the case of invasive carcinoma, growth into the underlying connective tissue, with or without the formation of corneal pearls (SOUSA SANTOS, 2018).

The three main forms of diagnostic confirmation are the search for the p16 protein by immunohistochemistry (IHQ), viral DNA in situ hybridization (HIS) and the polymerase chain reaction (PCR) test. The most conventional is the IHQ test, but for greater specificity HIS and PCR are used. HIS can be performed with both DNA and RNA. DNA HIS amplifies signals in molecular probes that bind to viral DNA, which enables differential nuclear detection and diffuse patterns of histological context of HPV-infected tumor cells. PCR is the most widely used method to detect and genotype HPV, it amplifies the messenger RNA signal at different magnitudes using pre-specific primers to detect the target viral DNA (QUREISHI *et al.*, 2017).

TREATMENT

Laryngeal papillomatosis is a difficult disease to treat due to resistance to various treatments and frequent recurrences. Various methods, including surgical approaches, topical and systemic drugs, have been tried, but with restrictions. Repetitive respiratory treatment aims to remove the lesions to ensure that the airway is open, as well as improving the voice. However, relapses are common; as treatment does not eliminate the virus (MARTHA *et al.*, 2013).

The treatment of HPV-associated SCC of the larynx generally follows the same guidelines for the treatment of non-HPV-related SCC, involving surgery, radiotherapy and chemotherapy. However, due to the greater sensitivity of HPV-positive tumors, there is a growing trend to consider less aggressive treatments for these patients in order to preserve laryngeal function and

improve quality of life (ANG *et al.*, 2010).

HPV, especially subtypes 16 and 18, is known for its ability to cause malignant transformations in epithelial cells. HPV infection in the larynx can lead to genetic mutations that promote the development of SCC. These cases often have a better prognosis than tobacco-related SCC, due to the greater sensitivity of HPV-induced tumors to radiotherapy and chemotherapy (GILLISON *et al.*, 2008).

In the 1970s, CO2 laser surgery was introduced to ablate lesions using thermal energy. Despite the positive results obtained with this technique, issues such as the intensity and time of exposure to the CO2 laser can lead to damage to the tissues adjacent to the lesion, chronic glottic edema and vocal fold scarring. In addition, it has been shown that HPV DNA particles can be aerosolized during the procedure. The microdebrider was first used as an alternative in the excision of papillomas in 1999, by Myer *et al*. This instrument has similar precision to the CO2 laser, has thinner blades with different angles associated with suction and irrigation. In addition, the microdebrider proved to be superior due to its lower cost, no risk of thermal trauma to the airway and less exposure to the spread of particles. Studies also infer a reduction in surgical time with the use of this instrument (MARTHA *et al.*, 2013).

Adjuvant therapies are being tested, including the antiviral cidofovir (an antiviral analog of cytosine, which interferes with the synthesis of viral DNA and prevents its replication), which has shown remission in cases of respiratory papillomatosis. The medication is applied to the patient under general anesthesia via direct laryngoscopy. It can be injected anywhere in the larynx without causing scarring or fibrosis. Its effect lasts from days to weeks, and a variable dosage may be indicated. While the use of cidofovir has promising potential to improve quality of life and reduce surgery, the lack of controlled studies prevents its routine use. Treatment with radiotherapy has been used as an alternative, but has been shown to increase the risk of malignant transformation (MARTHA *et al.*, 2013).

PREVENTION

The main means of prevention is sex education, with the use of condoms in all sexual relations, as protection against primary HPV infection and other STIs. Another form of prevention is the HPV vaccination scheme offered by the Unified Health System (SUS) (FEBRASGO, 2023). There are currently two HPV vaccines approved by regulatory bodies in Brazil: the quadrivalent vaccine, which provides protection against HPV types 6, 11, 16 and 18, and the bivalent vaccine, which only protects against the oncogenic types 16 and 18. The vaccine is made by genetic engineering, from particles similar to the viral capsid (VLP). These are encoded proteins devoid of viral genetic material, so they do not cause infection (CARDIAL *et al.*, 2019).

The main aim of vaccination is to prevent cervical cancer and other cancers, such as laryngeal and oropharyngeal cancer, caused by oncogenic HPV, in order to reduce the outcomes related to the disease, as well as genital warts caused by low-risk types. The vaccination coverage target is 80% of the target population (IWAMOTO *et al.*, 2017).

The immunity conferred by the vaccine is based on the production of antibodies against VLP, inactivating the virus and blocking epithelial infection. This mechanism is not effective if the patient has already acquired the infection, so the effectiveness of the vaccine is greater when applied before the beginning of the patient's sexual life (CARDIAL *et al.*, 2019).

Under the SUS, the vaccination schedule is indicated for girls and boys aged 9 to 14, in two doses at 0 and 6 months. For immunosuppressed patients (people living with HIV, transplant patients and cancer patients), from 9 to 45 years of age, the vaccine should be given in a 3-dose schedule, at 0, 2 and 6 months (FEBRASGO, 2023).

CONCLUSION

Laryngeal cancer has an etiology defined by the Human Papillomavirus in approximately 30% of cases. From this perspective, laryngeal squamous cell carcinoma accounts for the majority of laryngeal cancer cases. There is a lack of awareness among the population about the malignancy caused by HPV, which is a risk factor for late diagnosis of squamous cell carcinoma. This highlights the importance of early investigation and prevention in order to reduce morbidity**.**

REFERENCES

ANG, K. *et al.* Human papillomavirus and survival of patients with oropharyngeal cancer. **New England Journal of Medicine**, v. 363, n. 1, p. 24-35, 2010.

BETZ, S. HPV-Related Papillary Lesions of the Oral Mucosa: A Review. **Head and Neck Pathology.** 2019;13:80–90.

BUEXM, L. Carcinoma De Células Escamosas De Orofaringe Em Pacientes Do Inca: Características Sociodemográficas, Clínico-Patológicas E Moleculares. **Tese apresentada ao Instituto Nacional de Câncer como parte dos requisitos para a obtenção do título de Doutora em Oncologia**. – Rio de Janeiro, 2018.

BURLAMAQUI, J. *et al.* Human Papillomavirus and students in Brazil: an assessment of knowledge of a common infection-preliminary report. **Brazilian journal of otorhinolaryngology**, v. 83, n. 2, p. 120-125, 2017.

CARDIAL, M. *et al.* Papilomavírus humano (HPV): Aspectos da prevenção - Vacinas. In: **Programa vacinal para mulheres**. São Paulo: Federação Brasileira das Associações de Ginecologia e Obstetrícia; 2017. Cap. 4, p. 26-39. 2019.

CARVALHO, N. *et al*. Protocolo Brasileiro para Infecções Sexualmente Transmissíveis 2020: infecção pelo papilomavírus humano (HPV). **Epidemiologia e Serviços de Saúde**, v. 30, p. e2020790, 2021.

DE SOUSA SANTOS, R. Aspectos clínicos e histopatológicos de carcinomas espinocelulares orais: série de casos.

DERKAY, C. Recurrent respiratory papillomatosis. **The Laryngoscope**, v. 111, n. 1, p. 57-69, 2001.

DERKAY, C.; WIATRAK, B. Recurrent respiratory papillomatosis: a review. **The Laryngoscope**, v. 118, n. 7, p. 1236-1247, 2008.

DOGANTEMUR, S. *et al.* Assessment of HPV 16, HPV 18, p16 expression in advanced stage laryngeal cancer patients and prognostic significance. **Brazilian Journal of Otorhinolaryngology**, v. 86, n. 3, p. 351-357, 2020.

DUARTE, D. *et al.* "Prevalence Of Human Papillomavirus Infection and Cervical Cancer Screening Among Riverside Women Of The Brazilian Amazon". **Revista Brasileira De Ginecologia E Obstetrícia**, vol. 39, no. 07, 2017, p. 350-357.

EGAWA, N. *et al.* Human papillomaviruses: Epithelial tropisms and the development of neoplasia. **Viruses**. 2015.

FEDERAÇÃO BRASILEIRA DAS ASSOCIAÇÕES DE GINECOLOGIA E OBSTETRÍCIA (FEBRASGO). Informações sobre a Vacinação contra HPV. 2023.

FERLAY, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. **Int J Cancer**. 2015;136(5):E359-386.

GILLISON, M. *et al.* Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. **Journal of the National Cancer Institute,** 100(6), 407-420, 2008.

INSTITUTO NACIONAL DE CÂNCER (INCA). Ministério da Saúde. IST: saiba quais são os principais sintomas e formas de prevenção. 2023.

IWAMOTO, K.; TEIXEIRA, L; TOBIAS, G. Estratégia de Vacinação contra HPV. **Revista de Enfermagem UFPE Online**. v.11, n.12. 2017.

JOHNSON, D. *et al.* Head and neck squamous cell carcinoma. **Nat Rev Dis Primers**. ; 6(1): 92.-2021.

KASHIMA, H. *et al.* Sites of predilection in recurrent respiratory papillomatosis. **Annals of Otology, Rhinology & Laryngology**, v. 102, n. 8, p. 580-583, 1993.

KAYODE, A. The descriptive review, from recurrent respiratory papillomatosis of the disease, an enigmatic. **Arq. Int. Otorrinolaringol.** 2012;16(1):108-114.

LIMA, B. Infecções Sexualmente Transmissíveis (IST) e suas manifestações em boca. 2020. MARTHA, A. *et al*. Papilomatose laríngea. **Acta méd.(Porto Alegre)**, p. [6]-[6], 2013.

MARTINS, P.; PEREIRA, J. Métodos de diagnóstico da infecção pelo vírus do papiloma humano. Tese. Lisboa: Universidade de Lisboa; 2013.

MATOS, R. Análise do gene E6 de HPV de baixo risco em papilomatose de laringe. 2013.

MOSMANN, J. *et al.* Sexually transmitted infections in oral cavity lesions: Human papillomavirus, Chlamydia trachomatis and Herpes simplex virus. **J Oral Microbiol.** 2019;11(1):1632129.

MOUNTS, P.; KASHIMA, H. Association of human papillomavirus subtype and clinical course in respiratory papillomatosis. **The Laryngoscope**, v. 94, n. 1, p. 28-33, 1984.

MOURA, L. *et al.* **Cobertura vacinal contra o Papilomavírus Humano (HPV) em meninas e adolescentes no Brasil: análise por coortes de nascimentos**. 2019. Tese de Doutorado.

PEREIRA, V. *et al.* Carcinoma de células escamosas na laringe por infecção pelo papilomavírus humano. **Revista Contemporânea**, v. 4, n. 4, p. e3868-e3868, 2024.

QUREISHI, A. *et al.* Current and future techniques for human papilloma virus (HPV) testing in oropharyngeal squamous cell carcinoma. **European Archives of Oto-Rhino-Laryngology.** 2017 Jul;274:2675-83.

REIS, D. *et al.* Lesões orais do vírus hpv: revisão de literatura. **Revista Gestão & Saúde**, v. 22, n. 2, p. 19-28, 2020.

RIVERA, G.; MORELLI, F. Laryngeal papillomas. **StatPearls Publishing**; 2022.

SOHEILI, M. *et al.* Human papilloma virus: A review study of epidemiology, carcinogenesis, diagnostic methods, and treatment of all HPV-related cancers. **Medical journal of the Islamic Republic of Iran**, v. 35, p. 65, 2021.

SYRJÄNEN, S. Oral manifestations of human papillomavirus infections. **European journal of oral sciences**. 2018 Oct;126:49-66.

TRZCINSKA, A. *et al.* The Prevalence, Anatomic Distribution and Significance of HPV Genotypes in Head and Neck Squamous Papillomas as Detected by Real-Time PCR and Sanger Sequencing. **Head Neck Pathol** 2019;14(2):428–34.

VILLAGÓMEZ-ORTÍZ, V. *et al.* Prevalencia de infección por virus del papiloma humano en carcinoma espinocelular de cavidad oral, orofaringe y laringe. **Cir Cir.** 2016;84(5):363–8.

ZORZANELLI, L. *et al.* Câncer de laringe com HPV positivo: um estudo de caso. **Research, Society and Development**, v. 11, n. 7, e23611729936-e23611729936, 2022.