






IDENTIFICATION OF HUMAN PAPILLOMAVIRUS GENOTYPES IN JUVENILE LARYNGEAL PAPILLOMATOSIS. THREE-YEAR EXPERIENCE IN A CONCENTRATION HOSPITAL IN PUEBLA, MEXICO

IDENTIFICACIÓN DE GENOTIPOS DE VIRUS DEL PAPILOMA HUMANO EN PAPILOMATOSIS LARÍNGEA JUVENIL. EXPERIENCIA DE 3 AÑOS EN UN HOSPITAL DE CONCENTRACIÓN EN PUEBLA, MÉXICO

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ABSTRACT

Juvenile laryngeal papillomatosis (JLP) is a chronic benign disease of viral etiology, with an aggressive clinical course. In Mexico, the genotypes of the Human Papilloma Virus (HPV) that cause the disease have been poorly studied. The aim of this study was to identify HPV genotypes in PLJ patients. A descriptive and retrospective study was carried out, the records of patients with PLJ treated in a hospital in Mexico were reviewed, in the period 2018-2021. HPV was identified in all patients for genomes 6, 11, 16, and 18. nine patients were included, 56% women, mean age 9.5 ± 5.7 years; seven patients registered positivity for HPV-11 and 2 for HPV-6. The average age at diagnosis was 2.35 ± 1.77 years, with an average of 12 ± 11.56 surgical procedures. In conclusion, the most frequent genotypes in patients with PLJ were HPV-6 and HPV-11, with the latter predominating.

RESUMEN

La papilomatosis laríngea juvenil (PLJ) es una enfermedad benigna crónica de etiología viral, con un curso clínico agresivo. En México se han estudiado pobremente los genotipos del Virus del Papiloma Humano (VPH) que causan la enfermedad. El objetivo de este estudio fue identificar los genotipos del VPH en los pacientes con PLJ. Se realizó un estudio descriptivo y retrospectivo, se revisaron expedientes de los pacientes con PLJ atendidos en un hospital de México, en el periodo 2018-2021. En todos los pacientes se identificó el VPH para los genomas 6, 11, 16 y 18. Se incluyeron nueve pacientes, 56% mujeres, edad media $9,5 \pm 5,7$ años; siete pacientes registraron positividad al VPH-11 y 2 al VPH-6. La edad media al diagnóstico fue $2,35 \pm 1,77$ años, con promedio de procedimientos quirúrgicos de $12 \pm 11,56$. En conclusión, los genotipos más frecuentes en pacientes con PLJ fueron VPH-6 y VPH-11, predominando este último.

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INTRODUCTION

Juvenile laryngeal papillomatosis (JLP) is a chronic benign disease of viral etiology that occurs in childhood and adolescence^(1,2). Its prevalence in the United States is estimated to be around 1.8 per 100 000 adults and 4.3 per 100 000 children, with a higher frequency between two and four years of age. The total incidence is higher in patients with a low socioeconomic and educational level but not in severity^(2,3). Although laryngeal papillomatosis can present at any age, two age-related types have been described: juvenile (in children between one and four years of age and in adolescents) and adult (between 20 and 30 years of age). year old). This last type is less aggressive than the juvenile form⁽⁴⁾.

The etiologic agent of PLJ is the human papillomavirus (HPV). It causes benign proliferation of the squamous epithelium of the aerodigestive tract, associated with HPV genotypes 6 and 11, and the less prevalent 16, 18, 31, and 33⁽²⁾. In Colombia, a prevalence of genotypes 6, 11, and 16 are reported in adult papillomatosis⁽⁵⁾. In Mexico, genotype 6 has been reported as prevalent in up to 80% of cases⁽⁶⁾.

HPV infection is the most common sexually transmitted infection worldwide, with more than half of sexually active adults infected at some point in their lives⁽⁷⁾. Vertical transmission during birth occurs in the birth canal, which is the mechanism of transmission in children. Up to 50% of mothers of children with PLJ have HPV lesions on the genitals. The highest incidence of this variety is recorded in first-born patients and those obtained by delivery. Although cesarean delivery decreases transmission, it can occur in uterus in up to 12% of cases. In older patients, the infection can occur from oral sex practices^(1,2,4). The risk of vertical transmission in mothers with warts during childbirth is 1:231-400 cases. Other risk factors involved are a primigravid mother (due to a longer expulsive period), vaginal delivery, a male product, genetic and immunological factors, and the HPV genotype⁽⁸⁾.

Malignant transformation occurs in less than 1% of patients and has generally been reported in adults associated with another risk factor, such as smoking or radiation exposure, as well as in children with the extensive and prolonged disease with extensive dissemination; this type of malignancy has been

observed by the mutation of the p53 proto-oncogene⁽⁴⁾. HPV 11 infection in children appears to have a higher risk of airway obstruction and a higher probability of requiring a tracheostomy to maintain a patent airway⁽¹⁾.

Therefore, the objective of the present study was to identify human papillomavirus genotypes in patients with juvenile laryngeal papillomatosis at a concentration hospital in Puebla, Mexico.

METHODS

A descriptive, cross-sectional, retrospective study was carried out in a town in Mexico. The population consisted of patients diagnosed with PLJ, in a tertiary-level concentration hospital of the Mexican Institute of Social Security that cares for the population of the center-south of the country (states of Puebla, Tlaxcala, Veracruz, Oaxaca), the one with the lowest income. in Puebla, Mexico. Patients younger than 18 years of any age, of any sex, with a diagnosis of PLJ made by biopsy, during 2018-2021 were included. No patient was removed.

Age, gender, HPV genotype, age at diagnosis, number of surgical procedures, presence of tracheostomy, history of maternal papillomatous lesions, and route of birth were evaluated.

Biopsies from laryngeal lesions were processed within two months of taking the biopsy, with the following procedures: DNA extraction with the DNeasy Blood & Tissue kit (Qiagen®), HPV detection and typing by a polymerase chain reaction to 6, 11, 16, and 18 genomes using type-specific primer oligonucleotides. The amplification reaction was performed using the PCR-Master Mix kit (Promega®), and later the product was subjected to agarose gel electrophoresis to determine the presence or absence of the genotype.

This study was approved by the Local Health Research Committee number 2101 of the Mexican Social Security Institute. In each patient, the use of the sample was authorized by the parents or guardians with signed informed consent. Patient information was handled with strict confidentiality and was used exclusively for research purposes.





RESULTS

Nine patients were included, five women (56%) and four men (44%); with a mean age of 9.55 ± 5.7 years, three preschoolers (2-5 years), two schoolchildren (6-11 years), four adolescents (11-17 years). Genotype 11 was identified in seven patients (78%), and HPV 6 in 2 (22%). Genotypes 16 and 18 were not identified, nor were genotypes 6+11 coinfections. The age at clinical diagnosis was two months to six years, six patients in

the infant stage (two months-two years), two in preschool age (3-5 years), one school (six years). The mean was 2.3 ± 1.8 years. Regarding the number of surgical procedures performed at the time of evaluation, the average was 12 ± 11.6 procedures, a minimum of 2 and a maximum of 31. The predominance of genotype 11 is also reflected in the greater number of procedures, as shown in Figure 1.

BRIEF ORIGINALS

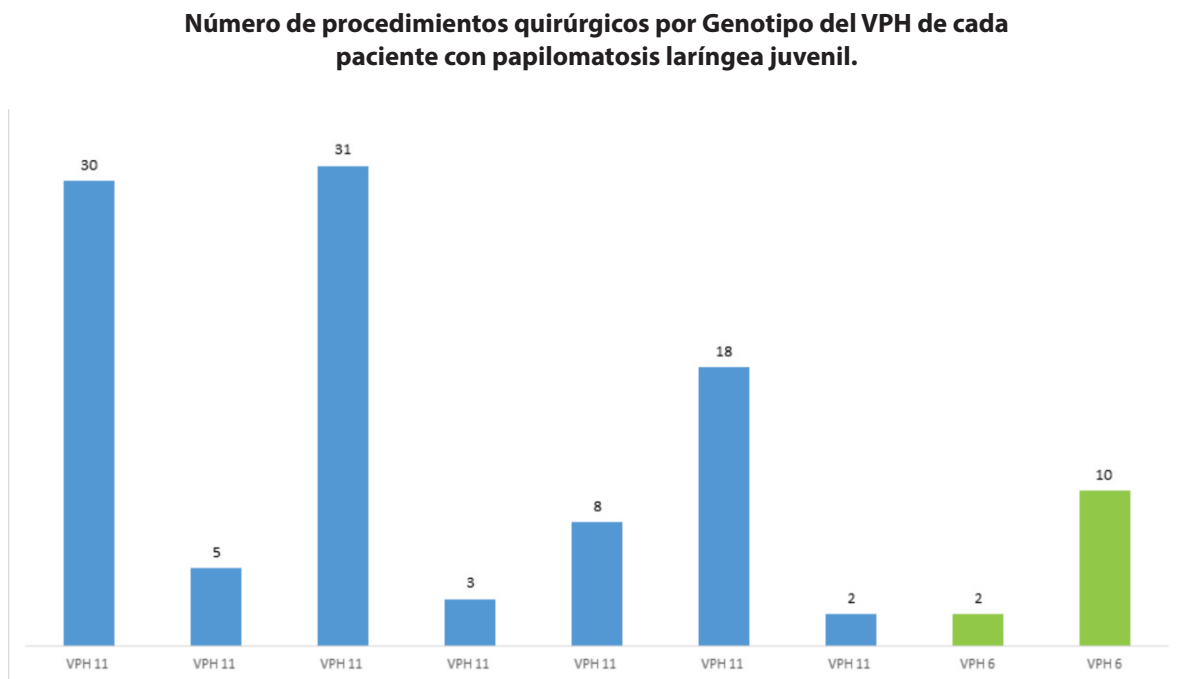


Figure 1. A number of surgical procedures and HPV genotype of each patient. The blue color corresponds to the patients with HPV-11 and the green color to the patients with HPV-6.

Tracheostomy was recorded in 5 patients (56%), 4 of them were positive for HPV-11 and one for HPV-6. All mothers reported absence of condylomatous lesions at birth; 8 patients were born by delivery, and one by cesarean section. The latter documented negative maternal vaginal cytology for HPV and orogenital transmission due to sexual abuse.

DISCUSSION

The human papillomavirus causes PLJ, and the most frequent genotypes are 6 and 11. This study sought to identify the genotypes that cause juvenile laryngeal papillomatosis in patients treated at a concentration hospital in Puebla, Mexico, in 2018-2021. PLJ is a chronic benign disease of childhood and adolescence,

whose natural history is highly variable and unpredictable. It can present from spontaneous remission, remain stable or even be highly aggressive, requiring frequent surgical treatments⁽¹⁾. Hoarseness is the first symptom, as papillomatous lesions usually present first on the vocal cords. This dysphonia usually goes unnoticed; stridor is the second symptom to present, first inspiratory and then biphasic. Other conditions are detected later, such as: chronic cough, recurrent pneumonia, dyspnea, dysphagia, developmental delay or respiratory distress. This may explain the variability in the age of diagnosis, according to the reported series.



The prevalence in this study throughout the pediatric age coincides with other series around the world, and detection was also made at an early age (less than six years). In comparison, most reports record primary diagnosis predominantly in children under ten years. Currently, the patients are mostly of school age and adolescents, while in other reports they predominate between nine and ten years⁽⁹⁻¹²⁾.

Studies report that the route of HPV transmission occurs through the vaginal canal during delivery⁽¹¹⁻¹³⁾. In our study, the route of delivery of most of the patients was by delivery; however, no maternal genital lesions were reported. In the case of the patient born by caesarean section, abuse and transmission through sexual contact are mentioned. Most of the viral typing works in the literature do not corroborate the history of maternal infection by clinical symptoms (condylomatous lesions), or by vaginal cytology. An intentional investigation in this regard in each case can be illustrative. There is also agreement on the slight predominance of the female sex⁽¹¹⁻¹³⁾, without achieving statistical relevance. Multicenter studies are required to corroborate this predominance.

In the present study, 100% HPV positivity was recorded in PLJ cases; however, other studies report a positivity to the virus between 67 and 98%⁽¹⁰⁻¹⁵⁾. It is noteworthy that virus detection was carried out two months after the biopsy was taken, which facilitates the extraction of viral DNA from the sample. Detection by polymerase chain reaction increases diagnostic sensitivity, explaining why all samples were positive⁽¹⁶⁾. Of the 200 HPV genotypes described since 1923, 16 and 18 are high risk and are found in the genitals or oropharynx. Genotypes 6 and 11 are the most frequent causes of airway pathologies. They are also responsible for 90% of genital condylomatous lesions, and are classified as

low-risk genotypes^(1,4,7). In this study, the prevalence of HPV was exclusive of genotypes 6 and 11, which coincides with several reports⁽¹⁰⁻¹⁵⁾. However, HPV-11 was the most prevalent, contrary to most studies, except the one by Gabbott et al.⁽¹⁴⁾, which also reports a higher prevalence of HPV-11. In the present study, not only the absence of genotypes 16 and 18 was reported, but also the absence of coinfections. The prevalence of up to 78% of genotype 11 in this population makes close surveillance necessary since it is related to greater disease severity⁽¹¹⁾.

Peñaloza-Plascencia et al. reported a high prevalence of coinfections in the western Mexican population (74%), with a very significant predominance of the HPV-16 genotype (85%). However, these discrepancies may be due to differences in the sensitivity of the method to detect the different viral genotypes and the DNA integrity of the samples analyzed. The higher prevalence of HPV-16 in the western region of Mexico⁽¹⁵⁾, and the predominance of HPV-11 in the center-south region (states of Puebla, Tlaxcala, and Oaxaca) should be verified in subsequent studies using similar detection methods.

PLJ can cause airway obstruction and is highly prone to recurrence. No treatment is effective in eradicating the disease⁽²⁾. The difficulty in eradicating respiratory tract lesions and frequent recurrence after surgical treatment cause an estimated cost of 150 million dollars per year in the United States^(1,5). The current standard of treatment is effective surgical resection of the lesions, either with microdebrider or laser. It has been suggested that this last modality carries a greater risk of transmission for the treating medical team and trans-surgical complications^(8,17). The removal or at least the





optimal reduction of the lesions to clear the airway must preserve the normal structures as much as possible to avoid complications such as glottic or subglottic stenosis. Other objectives should optimize vocal quality and increase the interval between surgical procedures^(1,4,8).

Papillomatosis is highly relapsing, so the number of surgical interventions in some patients is very high. The involvement in the maturity of the epithelium of the respiratory tract (with basal thickening and superficial nucleated cells in the stratified epithelium)⁽⁴⁾ also contributes to the need for repeated therapeutic intervention. The average number of interventions in this study is similar to that reported in other studies worldwide, and the presence of patients with more than 30 procedures is frequent. It stands out in this study that the highest percentage of patients with tracheostomy, and those with more than 15 surgeries were positive for HPV-11. Both findings confirm its invasive capacity and association with more severe evolutions⁽²⁾. Of course, studies, where lesion resection techniques are considered, could modify these figures.

Adjuvant treatments such as antivirals, biologics, retinoids, and the anti-HPV vaccine can modify the evolution, but more studies with a larger sample are required to define the benefit^(1,18-20). In the hospital where this study is based, we do not yet have adjuvant treatments, and only conventional cold surgical resection is performed by rigid suspension laryngoscopy under microscopic vision.

Among the limitations is the study methodology, which does not allow for establishing causal relationships or estimating risks. Although it is impossible to extrapolate the data to other populations, it allows for determining the prevalence of HPV infection to establish subsequent analytical studies.

In conclusion, in the cases of PLJ from central-southern Mexico, the predominant HPV genotypes are 6 and 11, with the latter more frequent. Multicenter studies are needed that include a larger number of patients and that consider various treatment modalities in addition to the investigation of maternal infection.

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