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Implication of low risk human papillomaviruses, HPV6 and HPV11 in laryngeal papillomatosis in Burkina Faso

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ABSTRACT

Purpose: Laryngeal papillomatosis is the most common benign tumor of the larynx of children. It is characterized by the development of exophytic proliferative lesions in the mucosa of the airways. Human papillomavirus (HPV) has been recognized as a causal agent among which HPV types 6 and 11 are the most frequently implicated. This disease affects the vocal cords and other important functions of the child. The difficulty of treatment is related to the high recurrence of papilloma growth after surgical removal. The objective of this study was to describe the implication of HPV6 and HPV11 in cases of laryngeal papillomatosis histologically confirmed in Ouagadougou.

Materials and methods: This was a descriptive cross-sectional study based on histologically diagnosed archival tissue; obtained in the last ten years (2007 to 2017) in the anatomy and cyto-pathology laboratories in Burkina Faso. These fixed and paraffin-embedded tissues were deparaffinized with xylene before HPV DNA extraction; then HPV6 and HPV 11 were identified by real-time multiplex PCR.

Results: The prevalence of low-risk HPV infection (HPV-LR) was 54.84% in histologically confirmed laryngeal papillomatosis in Ouagadougou. Among the HPV-LR positive samples, HPV6 and HPV11 genotype prevalence's were respectively 41.17% and 35.3% while the HPV6 / HPV11 co-infection was 23.53%.

Conclusions: The results show the implication of HPV6 and HPV11 in laryngeal papillomatosis in Burkina Faso with a high prevalence.

1. Introduction

Laryngeal papillomatosis is a benign tumor papillary type of squamous nature, usually seen in children. This pathology affects the vocal function and vital respiratory prognosis. It is located on the larynx but can extend from the back of the oral cavity or nasopharynx to the trachea or bronchi, to achieve a diffuse pharyngo-laryngo-tracheo-bronchial form. The typical manifestation is usually functional disorders with changes in the voice or cough, and secondary dyspnea that may include respiratory distress [1]. Well known clinically, its pathogenesis is still poorly understood, although the involvement of viral infection in the pathogenesis is now proven. Many clinical, epidemiological and molecular studies have demonstrated the causal role of human papillomavirus (HPV) infection in the development of laryngeal papillomatosis. Of the 120 genotypes of HPV infecting the genital and ORL tract genotypes 6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73 and 81 are at low risk. However, the HPV6 and HPV11 genotypes are 90% responsible for genital warts and condylomas of the larynx in children [2,3]. The genotype HPV 11 appears to be associated with more severe forms, with an increased risk of airway obstruction [4].

Treatment of laryngeal papillomatosis is essentially based on per-endoscopic pelts. Most of these surgeries are performed in the first years of life, with an average estimate of four operations per year [3]. The evolution process of laryngeal papillomatosis even after surgical treatment is still uncertain. Vaccination against HPV infection improves the prevention of the disease.

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In a study conducted by Sereme et al. [5] in Burkina Faso, several cases of laryngeal papillomatosis were detected in children from 6 months to 17 years treated and followed in the ORL department of CHU Yalgado between 2007 and 2012. This study focused on 36 cases and there were 33 cases of recurrence after treatment and 3 deaths. To date, no study has yet investigated the involvement of low-risk HPV6 and HPV11 papillomaviruses in laryngeal papillomatosis in Burkina Faso. Although since 2015 a Gardasil 4 vaccination campaign through the Global Alliance Program for Vaccines and Immunization (GAVI) has been initiated in the pilot districts of Solenzo and Baskuy.

The objective of the present study was to search for HPV6 and HPV11 in cases of histologically confirmed laryngeal papillomatosis in Ouagadougou.

2. Material and methods

2.1. Sampling and data collection

A descriptive cross-sectional study with retrospective data collection conducted a from 2007 to 2017. The Anatomy and Pathology Cytology Registries of the Yalgado Ouedraogo University Hospital (CHU-YO), the Shiphra Clinic, Sandof Clinic and the Philadelphia Clinic in Ouagadougou, Burkina Faso were consulted. During the last ten years corresponding to the study period, 47 cases of benign papillomatosis of the ORL field were diagnosed, including 31 cases corresponding to laryngeal papillomatosis, 12 cases of oral papillomas and 04 cases of nasal papillomas.

Paraffin blocks containing the archival tissues after histological diagnosis were cut with microtome in the Department of Anatomy and Cyto-Pathology of CHU-YO in Ouagadougou, Burkina Faso.

For each sample, we cut four or five sections of tissue with thickness inferior to 20 µm. But before this procedure the blocks were placed at −20 °C for 1 h. The resulting tissue sections were introduced into Eppendorf tubes nuclease-free after removal paraffin excess. The molecular analyzes were performed in the Molecular Biology and Genetics Laboratory (LABIOGENE, University of Ouaga I Prof. Joseph Ki-Zerbo).

2.2. Molecular analysis

2.2.1. Extraction of HPV DNA from fixed and paraffin-embedded tissues

DNA extraction was done using the FFPE DNA Purification kit (Norgen Biotek Corporation, Italy). Archived fixed and paraffin embedded tissue samples were deparaffinized with xylene prior to the extraction.

The samples were fixed in Bouin in most cases. The bouin is known to be a compound that can make immunohistochemistry impossible or inhibit PCR. Then the samples were treated with 10% PBS and the deparaffin protocol was thus modified as follows: One (1) ml of xylene was added to the sample, vortexed and incubated at 50 °C for 10 min then centrifuge at 14,000 RPM for 2 min. After centrifugation, the supernatant was removed and the step was repeated with xylene for a second time. After the xylene step, we added 1 ml of absolute ethanol to the sample, which was then vortexed and centrifuged at 14,000 RPM for 2 min. The supernatant was discarded and the ethanol step was repeated for a second time.

After xylene dewaxing, extraction of the HPV DNA consisted of the following steps: lysis of the cells, heating by incubation at 50 °C for 1 h, followed by 90 °C for 1 h to release the genetic material, DNA precipitation with absolute ethanol, DNA binding to the columns followed by three rounds of column washing and then elution of the DNA. This extraction was done following the protocol described by the manufacturer. The DNA extract thus obtained was stored in the freezer at −20 °C before PCR amplification.

2.2.2. Real-time PCR for low-risk HPV detection

The PCR amplification of the DNA extracted from the samples was carried out in order to search for human papillomaviruses. This PCR was done using SACACE biotechnologies® 'HPV Low Risk Typing Real™ V11-100FRT (6 and 11) kit which can detect the two low-risk HPV6 and HPV11 human papillomavirus genotypes. This PCR was done following the protocol described by the manufacturer.

2.3. Results interpretation

The study used 3 types of fluorescents: FAM/Green, JOE/Yellow, ROX/Orange. PCR is valid if positive controls and standards have a signal for all Fam, Joe, Rox fluorochromes and no signal for the negative control. The results were interpreted with the Microsoft Excel HPV Typing Program Real-Time Results Matrix.xls of the Low Risk Typing Real-TM' kit (Sacace Biotechnologies®, Italy) provided by the manufacturer.

2.4. Ethical considerations

This study was approved by the CERBA Research Ethics Committee and Saint Camille Hospital in Burkina Faso, by deliberation No. 2017/CERBA/II-24/0019. The confidentiality and anonymity of the information collected was respected.

2.5. Statistical analyzes

Statistical analyzes were performed using IBM SPSS statistical software and the chi-square test was used to compare the results. The results were considered significant for a p-value of less than 5%.

3. Results

3.1. Characteristics of the sample

Histologically, the samples taken this study showed a benign tumor proliferation, arranged in papillae. The appearance is suggestive of laryngeal papillomatosis. This retrospective study was conducted on 31 cases of laryngeal papillomatosis in a population aged between 04 months and 65 years with a median age of 13 years. The following parameters were studied: sex, age and prevalence of HPV6 and HPV11. In this study, all 31 formalin-fixed and paraffin-embedded biopsy tissue blocks, histologically diagnosed with laryngeal papillomatosis, gave a valid PCR result. Tissue blocks were collected from 15 male and 16 female patients with a sex ratio of 1.06.

We divided the study population into two age groups: children group [4 months–15 years] and adults group [28–65 years] taking into account the bimodal distribution of laryngeal papillomatosis [2,6]. The most represented age group was from children group (61.3%).

The most common symptoms in all patients were difficulty of breathing (100%), while some had coughing. Sixty-six (66)% of patients presented late (intense dyspnea). Instrumental peeling of papillomas was the only therapeutic method and was performed in all patients and more than half presented themselves in hospital after recurrence. A patient of 05 years old was at his fourth surgery. The diagnosis made by the clinician was varied, including asthma, laryngotracheo-bronchitis, aspiration of the foreign body and laryngomalacia.

Among patients, the larynx was the most affected part of the infiltrated airways of the papilloma, which crossed the different sub-regions of the glottis. Among these patients, 70% had vocal cord involvement, 25% had oral (pharynx, maxillary, gingival) involvement and 5% had nasal involvement.

3.2. Prevalence of HPV-LR infection

The results obtained show that 54.84% (17/31) of the analyzed tissue blocks were infected with HPV-LR. Among these samples carrying HPV-LR, the predominant genotype was HPV6 with a prevalence of

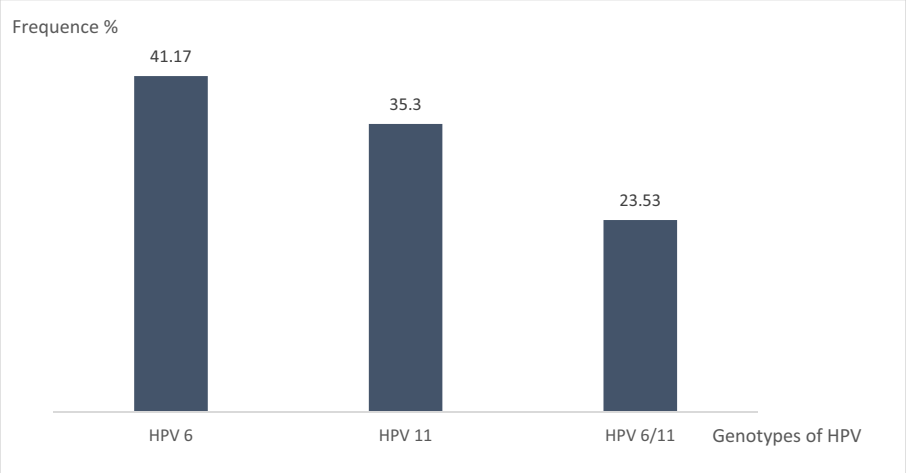

Fig. 1. Prevalence of HPV-LR genotypes among positive samples.

41.17% or 7/17 (Fig. 1).

3.3. Portage and distribution of HPV-LR genotypes by age groups

The Table 1 gives an indication of the carriage of HPV-LR per two age groups [4 month–15 years] and [28–65 years]. The rate of carriage was observed among [4 month–15 years] with a value of 64.28%. Regarding the distribution of genotypes, HPV11 and HPV 6 were found in all age group with equal predominance (29.41%). HPV6/HPV11 coinfection was only found in [4 month–15 years] as shown in Fig. 2.

4. Discussion

In laryngeal papillomatosis, the elective seat of papillomas is the endolarynx. The lesions usually start on the floor of the ventricles or on the vocal cords. During evolution, clumps of papilloma can extend to the entire larynx, progress in the hypopharynx and tracheobronchial tree. Sometimes papilloma grafts occur on the lips, nasal vestibule, posterior and free edges of the soft palate, or the tonsillar pillars [7].

In the present study, papillomas were located exclusively at the laryngeal level with vocal cord involvement in 70% of patients, oral (pharynx, maxillary, gingival …) involvement in 25% and nasal involvement in the remaining 5%.

HPV testing in the study samples showed a prevalence of 54.8%. Velyvyte et al. [8] have reported a higher prevalence of 88.9%. These authors worked on fresh specimens from the upper respiratory tract consisting of the nose, nasal cavity, mouth, pharynx (aerodigestive junction) and larynx in the throat. While in this retrospective study we worked only on laryngeal tissues fixed formalin and preserved in paraffin and archived over a decade. In addition, the difference in prevalence could be explained by the fact that in the study from Velyvyte et al. [8], other HPV genotypes in addition to HPV 6 and 11 was found.

Regarding the distribution of HPV genotypes, the prevalence of HPV6 was 41.17%. This was greater than the prevalence of HPV 11 (35.3%) and HPV6-11 co-infection (23.53%), but without a statistically significant difference (p = 0.085).

Table 1
HPV-LR carriage by age groups.

Age group	4 months – 15 years n (%)	28–65 years n (%)	Total n (%)
HPV-LR –	6 (35.72)	8 (50)	14 (45.16)
HPV-LR +	9 (64.28)	8 (50)	17 (54.84)
Total	15 (48.38)	16 (51.62)	31 (100)

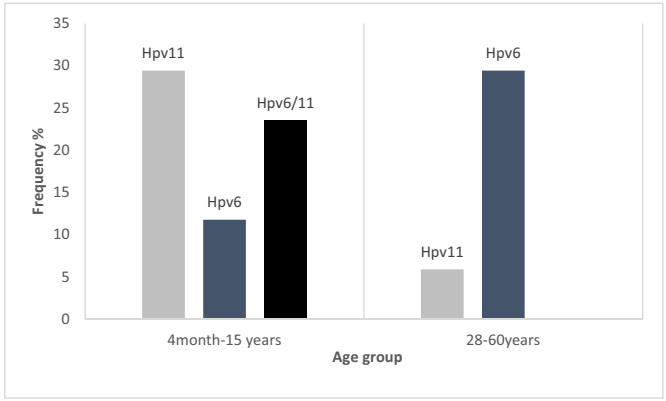

Fig. 2. Distribution of HPV-LR genotypes by age group.

Some authors [9] reported a similar distribution in Norway with predominance of HPV6 (64.25%) followed by HPV11 (19.32%) and HPV6-11 co-infection (7.2%). Mahnke et al. [10] also found HPV 6 (3/5) higher than HPV 11 (1/5). These authors who have worked on respiratory papillomatosis and juvenile papillomatosis have indicated that the HPV11 genotype associated with respiratory papillomatosis is considered more aggressive than the HPV6 genotype. In addition, HPV 11 is more often associated with extra laryngeal spread and increases the frequency of surgery.

Laryngeal papillomatosis is a condition usually seen in children and in this study, the median age was 13 years. The pediatric age indicated in this condition seems to be a well-recognized fact in several studies [6,11,12]. However, several authors [2,13,14] lean towards a bimodal distribution of the age of onset of the first symptoms; half of the cases beginning at childhood around 2–5 years old and the other half at adulthood around 20–30 years of age [2,6,7].

In this study, HPV infection was present in patients of all two predefined age groups. In the children group, 64.28% of the samples was infected by HPV compared to 50% of the adult group. HPV6-11 co-infection was found only in the first group with a prevalence of 23.52%. We observed a higher rate of HPV 11 (29.41%) infection in children than adults (5.88%). Other authors have reported a similar prevalence of HPV11 (28.6%) in children [9]. The clinical implication is not clear, but some have postulated a more aggressive clinical course in terms of more extensive growth of the papillomas and hence more frequent need of surgeries [15,16].

In general, the presence of HPV in the oral cavity or larynx could be due to oral sex practice according to Heck et al. [17]. Due to the

resistance of this virus, indirect transmission, under conditions of promiscuity, or any other contaminated surface is also possible.

In this study, the presence of HPV in samples in the children group could be explained by a probable transmission from mother to child at the time of delivery followed by the persistence of the virus. High levels of HPV DNA have been found in oral samples in infants with HPV-positive mothers immediately after delivery [18,19]. This transmission may cause laryngeal papillomatosis in children if the mother has anal or genital condyloma. An alternative to fight against laryngeal papillomatosis in children would therefore be to prevent HPV6 and HPV 11 infection in women since their childhood. This may involve vaccination against these genotypes, which can be given as early as 9 years of age; the vaccine is already available in Burkina Faso.

5. Conclusion

Our results show the involvement of a high prevalence of HPV6 and HPV11 genotypes in laryngeal papillomatosis in Burkina Faso. The evolution of the disease is marked by recurrence after treatment. Faced with this affection and the difficulty of surgical treatment, it would be necessary to adopt preventive policies and strategies for a better case management. Our results can therefore be taken into account in the vaccination strategy for scaling up in Burkina Faso.

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Declaration of interest

The authors declare that there is no potential conflict of interest.

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