


# Detection of Low-Risk and High-Risk Oncogenic Human Papillomavirus in Archived Tissues from ENT Tumors in Burkina Faso

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**How to cite this paper:** Kandé, A., Ilboudo, M., Ouermi, D., Traore, I.M.A., Ouattara, A.K., Zohoncon, T.M., Traore, E.M.A., Djigma, F.W., Obiri-Yeboah, D., Poaty, H., Gyebre, Y.M.C., Lompo, O.M., Ouedraogo, C. and Simpoire, J. (2023) Detection of Low-Risk and High-Risk Oncogenic Human Papillomavirus in Archived Tissues from ENT Tumors in Burkina Faso. *Journal of Biosciences and Medicines*, 11, 171-180.

<https://doi.org/10.4236/jbm.2023.119015>

**Received:** August 4, 2023

**Accepted:** September 18, 2023

**Published:** September 21, 2023

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## Abstract

Human papillomavirus (HPV) is classified into high-risk HPV (HR-HPV) and HPV (LR-HPV) according to their oncogenic potential. These viruses can be found in the cervix, vagina, vulva, anus and in the ENT sphere. HPV ENT infections can lead to benign or malignant tumors in which we could find both LR-HPV and HR-HPV genotypes. The objective of this study was to investigate the genotypes of HR-HPV and LR-HPV in archived tissue samples derived from both benign and malignant tumors of the ear, nose, and throat (ENT) in Ouagadougou, Burkina Faso. One hundred and twenty formalin-fixed, paraffin-embedded archived tissues of the ENT sphere from 26 benign tumors and 94 malignant tumors were included. The tissues were first deparaffinized with xylem. The extracted DNA was used to test for high-risk and low-risk HPV by Real-Time Multiplex PCR. HPV DNA was found in 57.7% (15/26) of benign tumors and 43.61% (41/94) of malignant tumors. The prevalence of HPV infection was 46.67% (56/120) in all tumors combined. The most common HPV genotypes found were HPV 11 (34.28%), HPV 6 (30%), HPV56 (14.28%) and HPV 33 (8.57%). There were 21.43% (12/56) cases of genotypes co-infections with 10 cases of double infection and 2 cases of triple infection. Both low-risk and high-risk HPV are found in ENT tumors with relatively high HPV prevalence.

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## Keywords

HPV, Archived Tissues, ENT, Tumor, Burkina Faso

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## 1. Introduction

Human papillomavirus (HPV) is a double-stranded circular DNA virus belonging to the papillomavirus family. Infection by HPV is one of the most common infections in the world [1]. These viruses are classified into high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) according to their oncogenic potential. The role of HR-HPV in the development of cervical cancer has been well demonstrated for many years [2] [3]. HPV infections are found in the cervix as well as the vulva, anus, vagina and ENT sphere [4] [5]. ENT infections are quite common and can lead to benign tumors but also to cancers. Papillomatosis is a benign tumor characterized by the proliferation of epithelial cells in clusters. This proliferation most often occurs in the larynx (laryngeal papillomatosis) and the nasal cavity (inverted papilloma, exophytic and oncocytic papillomas). About 75% to 85% of these benign lesions are found LR-HPV genotypes 6 and 114. Concerning malignant tumors such as ENT cancers, they are unfortunately discovered at late stages leaving little time for adequate care. The involvement of HR-HPV in ENT cancers has been recognized [6] [7]. It seems that the management of these HPV positive cancers is less complex, and they have a better prognosis compared to negative HPV ENT cancers. Depending on the type of tumor, the treatment calls for therapeutic modalities, combining lymph node tumor surgery, radiotherapy, and chemotherapy [8]. The identification of different HPV genotypes in ENT tumors could improve the prognosis and influence the therapeutic modalities. The 2008 National Comprehensive Cancer Network (NCCN) guidelines suggested HPV testing for oropharyngeal tumors [9]. Some countries like Burkina Faso in West Africa are not there for the moment. In this country, studies on archived biopsies tissues from ENT tumor reported in the registers of anatomy and cyto-pathology centers are helpful. A retrospective of these cases could guide on the types of HPV that are involved in ENT tumors in the country. So, this study focused on the diversity of HPV genotypes found in archived tissues from ENT benign and malignant tumors in the capital city, Ouagadougou. We hypothesized that both low-risk and high-risk HPV are found in ENT tumors regardless of type of tumors (benign or malignant). To test this hypothesis, HPV was investigated in the archived tissues and the frequencies of HR-HPV and LR-HPV were determined.

## 2. Material and Methods

### 2.1. Study Site, Type, and Population

We conducted a descriptive cross-sectional study with a collection of archived tissues over a 10-year period between 2007-2017 in four anatomy and cy-

to-pathology laboratories in Ouagadougou, Burkina Faso. The archived tissues fixed in paraffin blocks were from patients with a confirmed histological diagnosis of laryngeal papillomatosis or ENT cancers.

## 2.2. Sampling and Data Collection

This study was based on existing data from hospital registers recorded over a 10-year period. We have exploited the registers covering a decade in the retained centers to search the numbers for which papillomatosis and ENT cancers were histologically confirmed. From the registers, 120 sample numbers were selected for this study including 26 of laryngeal papillomatosis and 94 of ENT cancers. Data concerning patient's age, sex, location of infection and histological type of cancer were available. Localization of ENT Cancers was larynx, palate, maxillary, nasal, pharynx, Lip, ear, tongue and tonsil. The histological types of ENT cancer reported in the registries were: carcinoma, lymphoma, sarcoma, melanoma and cylindroma. In this study, we have combined all types of cancers in the group "malignant tumors" and all papillomatosis in the group "benign tumors" for analysis.

The selected numbers from registers were used to search for the corresponding blocks which were cut by microtome, to obtain sections of thickness  $\leq 20 \mu\text{m}$  for each sample. The samples were introduced on nuclease-free Eppendorf tubes and transported to the Molecular Biology and Genetics Laboratory (CERBA/LABIOGENE), Joseph KI-ZERBO University for molecular analysis.

## 2.3. Dewaxing of the Tissues

All the tissues were first treated with 10% PBS. Then 1 mL of xylene was added to the sample, vortexed and incubated at 50°C for 10 minutes and centrifuged at 14,000 RPM for 2 minutes. The supernatant was removed, and the xylene step was repeated a second time. Finally, 1 ml of absolute ethanol was added to the sample, which we then vortexed and centrifuged at 14,000 RPM for 2 minutes. The supernatant was discarded, and the ethanol step was repeated a second time.

## 2.4. HPV DNA Extraction

The NORGEN FFPE DNA Purification kit was used for HPV DNA extraction according to the manufacturer's protocol. We proceeded with the cell lysis step, followed by incubation at 50°C for 1 hour and 90°C for 1 hour to release the genetic material. The DNA was precipitated with absolute ethanol, washing of the columns and elution of the DNA were performed. The DNA obtained was stored at -20°C until amplification by PCR.

## 2.5. Real-Time Multiplex PCR

Extracted DNA was amplified by multiplex RT-PCR with the SaCycler-96 Real Time PCR v.7.3 (Sacace Biotechnologies, Italy) using two kits and following the protocol described by the manufacturer.

The detection of low-risk HPV was carried out using the “HPV low Risk Typing Real™ V11-100FRT (6 and 11)” PCR kit from SACACE biotechnologies®. This kit permits the detection of two low risk HPV genotypes (HPV 6 and HPV 11). The detection of high-risk oncogenic HPV in the DNA extracts of this study was carried out using the HPV kit “High Risk Typing Real-TM” from SACACE biotechnologies®. This Kit makes it possible to detect fourteen genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The PCR program used was as follows: 1 cycle at 95°C for 15 minutes; 5 cycles of 95°C for 05 seconds, 60°C for 20 seconds and 72°C for 15 seconds; 40 cycles of 95°C for 05 seconds, 60°C for 30 seconds and 72°C for 15 seconds. The results were interpreted with the Microsoft Excel “HPV Typing Real-Time Results Matrix.xls” program from the “High Risk and low risk Typing Real-TM” kit (Sacace biotechnologies®, Italy) supplied by the manufacturer.

## 2.6. Statistical Analysis

Statistical analyses were performed using IBM SPSS statistics 20 software and the chi-square test was used to compare results. The results were considered significant for a p-value of less than 5%.

## 2.7. Ethical Considerations

The study was approved by the Health Research Ethics Committee (CERS) of Burkina Faso: “Deliberation n°2022-03-066 of March 02, 2022”. The confidentiality of the data collected has been respected.

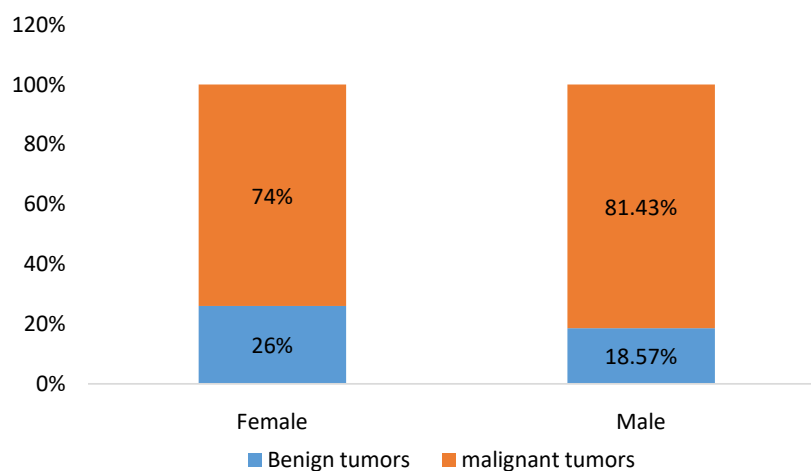
## 3. Results

### 3.1. Characteristics of the Included Samples

This study included one hundred and twenty (120) ENT tumor tissues with histological diagnosis revealing 94 cases of malignant tumors and 26 cases of benign tumors. Among tissues from cancers, the most frequent histological type reported in registries was carcinoma with 77.65% (73/94), followed by lymphoma 11.70% (11/94), sarcoma 4.25% (4/94), melanoma 3.20% (3/94) and cylindroma 3.20% (3/94). Tissue blocks were collected from 70 male and 50 female patients with a sex ratio of 1.4; patients' age ranged from 2 to 69 years. For female, they were 74% (37/50) cases of malignant tumors compared to 81.43% (57/70) cases among male (**Figure 1**). The difference between type of tumors in male and female was not statistically different (p value = 0.33).

### 3.2. Prevalence of HPV Infection

A valid PCR result was obtained with all 120 archived paraffin-fixed tissue blocks included in this study. The prevalence of HPV was respectively 57.7% (15/26) and 43.61% (41/94) in benign tumors and malignant tumors (**Table 1**). The combined prevalence of HPV infection regardless of the tumor type was 46.67% (56/120). Of the 16 HPV (14 HR-HPV and 2 LR-HPV) genotypes tested,



**Figure 1.** Distribution of samples according to gender and type of tumors.

**Table 1.** Presence of HPV according to the type of tumor.

Type of tumor	HPV+ n (%)	HPV- n (%)	Total n (%)
<b>Malignant tumors</b>	41 (43.61)	53 (56.39)	94 (78.33)
<b>Benign tumors</b>	15 (57.70)	11 (42.30)	26 (21.67)
<b>Total</b>	56	64	120 (100)

10 genotypes were found. The number of genotypes found in each sample varied from 1 to 3. Considering multiple infections, 70 HPV genotypes were detected in the whole population. The most frequent genotype was HPV 11 (34.28%) followed by HPV 6 (30%), HPV56 (14.28%) and HPV 33 (8.57%) (**Figure 2**).

### 3.3. Distribution of HPV Genotypes in Co-Infected Samples

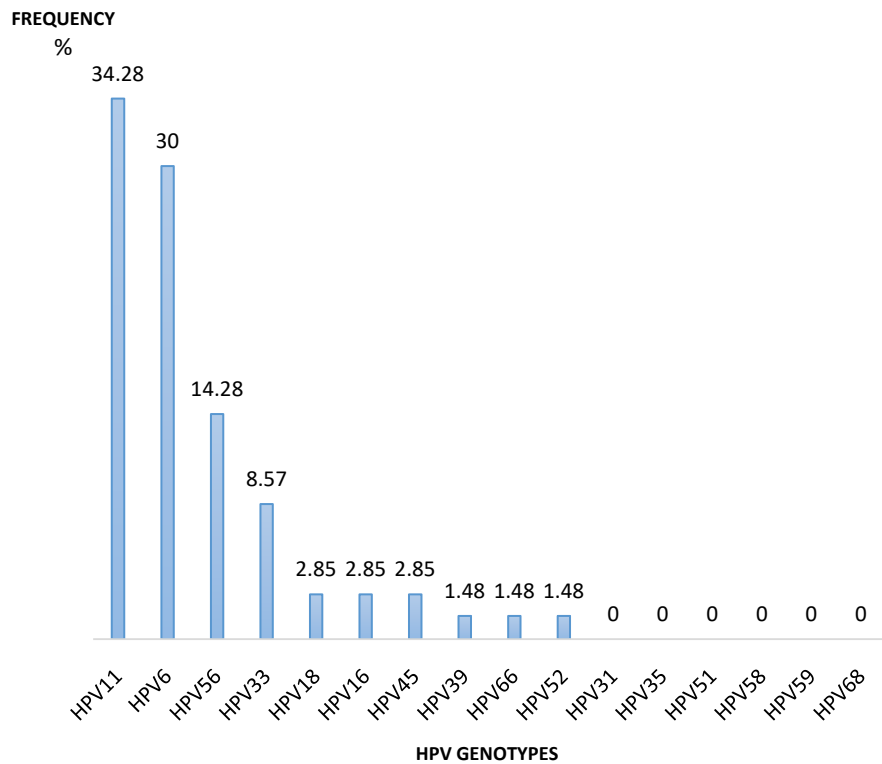
Among the HPV positive samples, there were 12 cases of co-infections 21.43% (12/56) regardless of HPV genotypes with 10 cases of double infection and 2 cases of triple infection. HPV 33 was the most common genotype in the different co-infections. There were 2 cases of co-infection between HR-HPV genotypes 16.6% (2/12), 5 cases of co-infection between LR-HPV genotypes 41.7% (5/12) and 5 cases of co-infection between LR-HPV and HR-HPV genotypes (**Table 2**).

### 3.4. Distribution of HR-HPV and LR-HPV in Tumors

Among the 56 samples positive to HPV, 15 samples were from benign tumors while the remaining 41 were from malignant tumors. **Table 3** shows that the HR-HPV were found in 13.33% (2/15) of benign tumors versus 48.8% (20/41) of malignant tumors ( $p = 0.08$ ). As for LR-HPV, they were present in 86.7% (13/15) of benign tumors against 51.2% (21/41) of malignant tumors with a significant  $p$  value ( $p = 0.02$ ).

## 4. Discussion

This study focused on ENT tumors tissues archived in anatomy and cyto-pathology



**Figure 2.** Prevalence of HPV genotypes in samples.

**Table 2.** Distribution of HPV genotypes in co-infected samples.

HPV genotypes co-infection		Number of cases
Between LR-HPV	HPV 6 - HPV 11	5
Between HR-HPV	HPV 33 - HPV 18	1
	HPV 45 - HPV 56	1
Between HR-HPV and LR-HPV	HPV33 - HPV 11	1
	HPV 33 - HPV 6	1
	HPV 66 - HPV 11	1
	HPV 16 - HPV 33 - HPV 11	1
	HPV 56 -HPV6 - HPV 11	1
Total		12

**Table 3.** Repartition of LR-HPV and HR-HPV according to type of tumor.

Tumors	LR-HPV n (%)	HR-HPV n (%)	Total
Benign tumors	13 (86.7)	2 (13.3)	15
Malignant tumors	21 (51.2)	20 (48.8)	41
Total	34	22	56

centers in Ouagadougou, Burkina Faso over a period of 10 years. In this country as elsewhere, the ENT pathologies are generally infections, trauma, tumors, and

malformations. In recent years, tumors are increasingly frequent, whether benign or malignant 9, the cases being seen in both men and women. The sex ratio in this study was 1.4 in favor of men. Some authors have described oral cavity cancers as a predominantly female disease in Ouagadougou, Burkina Faso [10]. In the present study, the frequency of malignant tumors was higher in male (81.43%) than in female (74%). Our results were similar to those reported in the literature about the male predominance in oral cavity cancers [11]. According to the authors of the study carried out in Ouagadougou, their results could be explained by poor oral hygiene more common in women than in men in Burkina Faso [12]. For this study, we only had the information found in the registers, which did not allow us to analyze many socio-demographic characteristics of the patients. However, the frequency of malignant tumors was not different between women and men.

This study reports a general prevalence of 46.67% (56/120) in ENT tumors. This prevalence is close to that of 46.5% found in oropharyngeal carcinomas in France [13] and 45.9% found in Japan [14]. However, it remains lower than the 71% obtained in the United States [15] and higher than several studies in Africa including the one that reported a prevalence of 3.4% in Senegal [16] and 19.23% in Ghana [17]. Among all HPV genotypes tested in the present study, 8/14 HR-HPV and 2/2 LR-HPV were found. The 4 most frequent genotypes were HPV 11, HPV 6, HPV 56 and HPV 33. The predominance of LR-HPV in the samples included in the present study is not really surprising. Indeed, the LR-HPV 6 and 11 are recognized as the genotypes found in laryngeal papillomatosis [18] corresponding to the group “benign tumors” in this study. HPV types 16 and 18 are globally responsible for 85% of cancer of the head and neck [19]. For the present study, HPV 16 and HPV 18 were classified as 5th most frequent with the same frequency of 2.6%. This distribution follows the same pattern as many HPV studies on cervical samples in Burkina [5] [20] [21] [22] which showed that HPV 16 and 18 were not the most frequent as elsewhere in the world.

In this study, multiple infections accounted for 21.43% (12/56) among the HPV positive samples. In general, co-infections between the different types of HPV are frequent and an HPV infection can be associated with HR-HPV and LR-HPV. Co-infections between HR-HPV were less frequently accounted for (16.6%) compared to LR-HPV co-infections (41.7%) and LR-HPV/HR-HPV co-infections (41.7%).

It is known that the pathologies associated with HPV vary depending on the genotype involved. LR-HPV is not associated with the development of cancer, but malignant transformation can occur if the pathology is also associated with HR-HPV [23]. Given that HPV transmission can be oro-sexual [24], this could explain the presence of high-risk genotypes in the ENT sphere. For the present study, the HR-HPV was found in both benign and malignant tumors with respectively frequency of 13.33% (2/15) and 48.8% (20/41). In general, benign tumors (in this case laryngeal papillomatosis) do not become cancerous [25] but

the presence of HR-HPV should lead to more monitoring of affected patients. Depending on the type of lesion and the location, different types of treatment are recommended, including surgical excision. However, no therapeutic method allows for the eradication of the virus. This is explained by the fact that the virus can persist lesions. It is therefore crucial to focus on hygiene and vaccination in the fight against HPV induced ENT tumors.

A nonavalent vaccine is available and directed against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58. It covers 6 genotypes of HR-HPV and the two genotypes of LR-HPV encountered in our study. In Burkina Faso, the use of the nonavalent vaccine could be expected to prevent certain HPV induced ENT tumors.

Despite the results obtained, this study has a number of limitations. One of the main limitations of this study was the lack of information on the habits and behaviors of the patients whose tissues were included. This did not allow for a discussion of the socio-demographic characteristics. In addition, the sample size was small due to the lack of registration of tumor cases in the country's health facilities.

## 5. Conclusion

Both low-risk and high-risk HPV are found in archived tissues from ENT tumors in Ouagadougou, Burkina Faso. The HPV prevalence obtained were relatively high reporting the involvement of these viruses in ENT tumors. These kinds of data about the repartition of HPV types may be useful to tailor vaccination and intervention strategies.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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