# Molecular Characterization of Human Papillomaviruses Associated with Cervical Cancer in Brazzaville, Congo

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## ABSTRACT

*Knowledge of the viral ecology of human papillomaviruses is essential in the prevention of cervical cancer. This work aimed to identify human papillomavirus genotypes associated with cervical cancers in the capital city of Brazzaville. 66 paraffin blocks of cervical biopsies were collected between 2016-2017. After extraction of viral DNA, amplification and genotyping was performed using Gene Xpert technology from the Xpert HPV kit. The mean age was 54.48 ± 16.98 years (range 18-88 years). HPV viral DNA was identified in all samples analyzed, giving a prevalence of 100%. The most frequent genotype was HPV-16 (80.3%). Six percent of the patients were carriers of the HPV-18/45 combination and 12.1% of other HPV-HR genotypes. HPV-16/HPV-18/45 co-infection was observed in 1.5% of cases.*

*According to the type of cancer induced, HPV-16 was found in 83.3% of squamous cell carcinomas and 50% of adenocarcinomas.*

*In this work, the authors observed a very high frequency of HPV-16 whatever the type of cancer in Brazzaville.*

## Keywords

Molecular characterization, HPV infection, Cervical cancer, Brazzaville.

## Introduction

Cervical cancer is the second most common cancer among women worldwide and accounts for about 10% of all cancers. In 2018, there were about 569,847 new cases of cervical cancer worldwide or 6.6% [1]. The estimate for Africa shows that 99038 new cases of cervical cancer were recorded in 2012 [2]. The prevalence of cervical cancer in sub-Saharan Africa is 10 to 20 times higher than in most European and East Asian countries [3]. In Congo, a study on the determination of the incidence of cancers in Brazzaville over the period 2016 to 2017, reveals that cervical cancer was in second place after breast cancer [4]. With 310 new cases reported in 2012 [5]. In 2017, according to the Brazzaville cancer registry, the age-standardized incidence of cervical cancer is 15.3 per 100,000 inhabitants, and that of breast cancer is 25.2 per 100,000 inhabitants [4]. Cervical cancer is the second leading cause of cancer death in women worldwide and the leading cause of cancer death in Africa; more than 270,000 women die from cervical cancer each year and more than 85% of them are from low- and middle-income countries [6]. In developed countries, the five-year survival rate is about 66%. [7]. In Africa, less than 50% of women with cervical cancer survive beyond five years. In the Congo, cervical cancer is the second most common cause of death among Congolese women, with 157 deaths per year per 100,000 women [5]. HPV is responsible for 99% of cervical cancer cases, which is the leading cause of cancer mortality in Africa. They are the most common viruses in sexually transmitted infections [8]. A meta-analysis of nearly 158,000 women with normal cervical cytology indicated an overall prevalence of 10.4%. The highest

prevalences (>20%) were observed in Africa. [9]. According to the WHO, approximately 1.4 million women worldwide are affected by cervical cancer, the majority of which are affected by types -16 and -18 [10]. Although most HPV infections are transient and disappear within two years without treatment [9], high-risk HPV types can cause persistent infection and are significantly associated with high-grade cervical lesions and cancer [11]. In addition, the primary prevention of HPV infection is through prophylactic vaccination. The 9-valent vaccine Gardasil-9® (Merck & Co. Inc., New Jersey, USA) targets the seven HR-HPV genotypes predominantly isolated in cervical cancer (HPV-16, -18, -31, -33, -45, -52 and -58) and the two predominantly isolated low-risk (LR) HPV (HPV-6 and HPV-11) [12]. Although the distribution of high-risk types in high-grade lesions (HSIL) and cancers varies little from one region of the world to another [13-15], a thorough understanding of these types is essential for the introduction of an effective vaccination program and for better monitoring of the viral ecology before and after vaccination in a population. This information is also necessary to assess the benefit of the vaccine on cervical cancer prevention in the population. Thus, the objective of this study was to characterize the human papillomavirus genotypes associated with cervical cancer in Brazzaville.

## Methods

### Collection of samples

This was done at the University Hospital of Brazzaville in the Anatomy and Cytopathology Department. After consultation of the registry, seventy (70) paraffin-embedded biopsies of cervical cancer were identified between 2016-2017. After review for confirmation of the diagnosis in the registry, sixty-six (66) biopsies were retained and included in this study. Notification of the diagnosis of cervical cancer and availability of the biopsy specimen were the two inclusion criteria for this study. All patients were between the ages of 18-88 years.

### DNA extraction

5 μm paraffin sections were taken on a microtome and stored in 1.5ml eppendorfs tubes. After conditioning, the samples were transported to Pointe-Noire to the virology and molecular biology laboratory of the Marie Madeleine GOMBES Foundation polyclinic.

 - Pre-treatment of samples: Sections were dewaxed with xylene and washed with 70% ethanol at room temperature. A final wash with PBS was performed for each sample before enzymatic digestion.

 - Enzymatic digestion: was performed using the lysis solution of the RNA/DNA Purification Kit (NORGEN, BIOTEK, CORP, Canada) mixed with 10μg/ml proteinase K.

 - DNA purification and elution: after several centrifugations and washes, the DNA was eluted in 100 μL of elution solution and stored at -20°C before use.

After extraction, a quantitative assessment of the DNA contained in each extract was performed by DNA assay using Qubit 3.0 fluorescence technology (Qubit® 3.0 Fluoremeter, life technology).

This assay allowed us to assess the concentration of DNA in ng/ml in each sample in order to adjust the PCR.

### HPV-DNA detection and genotyping

HPV type identification was performed by real-time PCR using GeneXpert technology (CEPHEID, USA) from the Xpert® HPV kit. The Xpert HPV Assay is an automated test for the qualitative detection and differentiation of HPV DNA by clustered molecular typing of oncogenic HPV. This is achieved by differentiating the presence of an HPV-16 genotype alone, by pooled genotyping of the -18/45 types and by pooled detection of other high-risk HPVs other than the -16, -18 and -45 types. The Xpert HPV assay cartridge was filled with 1mL of the DNA and DNAase-free Molecular Biology Water mixture.

### Statistical analysis

A database was created using Excel 2013 and statistical analysis was carried out using Epi info V7. The results were expressed as mean ± standard deviations for quantitative variables and as number and/or percentage for qualitative variables. The p-value indicated a statistically significant difference when its value was less than or equal to 0.05 (p ≤ 0.05).

## Results

### Histological type of cancer and age

Table 1 shows the distribution of histological types of cancer according to the age of the patients. Patients aged between 35-65 years accounted for more than 50% of cervical cancers regardless of histological type.

**Table 1:** Distribution of Histological Types of Cancer According To Age.

| Age (years) | Squamous cell carcinoma n (%) | Adenocarcinoma n (%) |
|---|---|---|
| 54.48 ans ± 16.98 ans* | 55.7 ± 15.21 ans* | 42.33 ± 17.21ans* |
| ≤ 34 | 5 (8.3%) | 2 (33.3%) |
| 35-65 | 42 (70%) | 3 (50%) |
| > 65 | 13 (21.7%) | 1 (16.7%) |
| Total | 60 (100%) | 6 (100%) |

### HPV Prevalence and Genotyping

Carriage of HPV infection was 100% (66/66) of cases. HPV-16 was in the majority with 80.3%. All results are reported in table 2.

**Table 2:** Prevalence of HPV types in the study sample.

| HPV type | Number (n) | Frequency (%) |
|---|---|---|
| HPV 16 | 53 | 80.3 |
| HPV 18/45 | 4 | 6 |
| Other HPV-HR | 8 | 12.1 |
| Co-infection HPV 16 & HPV 18/45 | 1 | 1,5 |
| Total | 66 | 100 |

### Correlation of genotypes and histological types

In our study, 83.3% of squamous cell carcinomas and 50.0% of adenocarcinomas were HPV-16 positive. The HPV-18/45 pair was identified in 33.3% of adenocarcinomas. Only squamous cell carcinomas had HPV-16/HPV-18/45 co-infection (Table 3).

**Table 3:** Correlation of Genotypes and Histological Types.

| HPV type | Squamous cell carcinoma | Adenocarcinoma |
|---|---|---|
| | n=60 (%) | n=6 (%) |
| HPV 16 | 50 (83.3) | 3 (50.0) |
| HPV 18/45 | 2 (3.3) | 2 (33.3) |
| Other HPV-HR | 7 (11.6) | 1 (16.6) |
| Co-infection HPV 16 & HPV 18/45 | 1 (1.6) | 0 (0.0) |
| Total | 60 (100) | 6 (100) |

P=0.0284

## Discussion

Our study was based on archived samples from 2016 to 2017 of paraffin blocks. These tissue types represent a potentially useful resource for retrospective epidemiological studies; it allowed us to identify high-risk genotypes associated with cervical cancers with CEIPHEID Gene Xpert technology. This technology is based on the principle of real-time PCR. Einstein et al., Castle et al., Heather et al. in Malawi, Akaaboune et al. in Cameroon respectively used the same technique [16-19]. The patients received in this laboratory come from different departments of the country and represent broad social and economic strata. Therefore, the results of our study may be of particular interest in the country. In this study, the mean age of the patients was 54.48 ±16.98 years with a peak between 35-65 years. This average is close to that observed by N'Guessan et al. in Côte d'Ivoire who reported an average age of 48.5 years with a peak between 41 and 50 years [20]. This observation supports the view that cervical cancer occurs at a relatively young age in Africa compared to Western countries [21,22]. The early onset of cervical cancer in Africa seems to be related to the increase in risk factors such as poor socio-economic conditions, early sexual debut, multiple sexual partners, and frequent childbearing [20].

The number of squamous cell carcinomas was about 10 times higher than adenocarcinomas. The highest number of squamous cell carcinomas was found in the age group 34-65 years. Bado Prosper et al. in Burkina had similar results (squamous cell carcinomas were 6 times higher than adenocarcinomas) [23]. These observations are reported by almost all others who have addressed these aspects in the literature [24,25]. We detected HPV-HR DNA in 100% of the samples analyzed. These results are close to the HPV detection rate of studies conducted in Morocco by Khair et al., in biopsies (92%), Siriaunkgul et al. in Thailand (96.9%) and GUL in Pakistan which notes 88% in cervical tissues preserved in paraffin by [26-28].

We report a frequency of HPV-16 of 80.3% and HPV-18/45 of 6%. Other HPV- HR and HPV-16 and -18/45 co-infections were respectively found in 12.1% and 1.5% of cases. Our results corroborate those found by most authors in the literature. Indeed, Naucler et al., Abate et al., Boumba et al. respectively identified HPV-16 as the most prevalent type in 56%, 52.5% and 82.5% of cases [29-31].

Pooled genotyping using Genexpert technology did not allow the identification of the other HPV-HR genotypes, which represent 12.1% of cases in our study. On the other hand, the HPV-18/45 pair represents only 6% of the cases in our study whereas these genotypes are the second most common after type 16. This genotypic profile would be slightly different from the global profile of which the most prevalent, according to the International Agency for Research on Cancer, are HPV types -16, -18, -45, -31, and -33 in high-grade infections, other studies have identified genotypes -16, -18, -35, -45 common to certain African countries [11,32,33]. Indeed, it has been reported that the distribution of genotypes in Africa is different from that observed in other regions of the world [9,34]. These authors observed that in Africa and North America HPV-16 was more prevalent followed by HPV-52, -58 and HPV-52, -53 respectively [34]. The HPV-16 profile followed by HPV-33 and HPV-31 identified by Boumba et al. could be found in our study if the genotyping technique used allowed it, as the populations were similar [31].

According to histological type, HPV-16 was identified in 83.3% of squamous cell carcinomas and 50% of adenocarcinomas. HPV-18/45 was identified in 3.3% of cases in squamous cell carcinomas and in 33.3% of cases in adenocarcinomas. Our results corroborate perfectly with the observations of the literature, according to which type 16 is preferentially identified in squamous cell carcinomas whereas type 18/45 in adenocarcinomas [35,36].

On the other hand, we note differences with some African studies, in particular that of Boumba et al. (Congo) and Ouédraogo et al. (Benin) [31,37]. We believe that these differences are methodological and due to the origin of the samples used.

In sum, the present study established the genotypic profile of HPV in the city of Brazzaville on paraffin-embedded biopsies with a histologically confirmed diagnosis of cervical cancer. Despite the small sample size, a larger study of cancer cases on fresh biopsies is desirable to better illustrate this genotypic distribution.

## Conclusion

The result of this study showed that the HPV- 16 genotype was the most frequent in squamous cell carcinomas, which represent more than 95% of cervical cancers. This study contributes to the knowledge of HPV infections in Brazzaville and can be used to implement a better policy to fight this pathology in terms of both prevention and management. These results also indicate that vaccination against HPV could be beneficial among the Congolese population.

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