



## Molecular Signature of Human Papillomavirus in Upper Aerodigestive Tract Cancers in Congo-Brazzaville

Anicet Luc Magloire Boumba<sup>1,2,3\*</sup>, Sylvain Diembi<sup>1,4</sup>, Boris. Otouana<sup>1,5</sup>,  
G. C. Gouoni<sup>1,6</sup>, A. B. Ecokounda Okoko<sup>1,6</sup>, F. Itiere Odzili<sup>1,6</sup>,  
Jean Felix Peko<sup>1,6</sup> and Gottran Ondzotto<sup>1,6</sup>

<sup>1</sup>Faculté des Sciences de la Santé, Université Marien NGOUABI, BP: 69, Brazzaville, Congo.

<sup>2</sup>Hôpital Général de Loandjili, BP: 812, Pointe-Noire, Congo.

<sup>3</sup>Zone de Recherche de Pointe-Noire, Institut de recherche en Sciences de la Santé, IRSSA, Brazzaville, Congo.

<sup>4</sup>Hôpital Général Adolphe SICE, Pointe-Noire Congo.

<sup>5</sup>Hôpital de Référence de Talangai, Brazzaville, Congo.

<sup>6</sup>Centre Hospitalier et Universitaire de Brazzaville BP: 32, Brazzaville, Congo.

### Authors' contributions

This work was carried out in collaboration among all authors. Author ALMB designed the study, performed the statistical analysis and wrote the protocol. Authors ALMB, SD and BO wrote the first draft of the manuscript. Authors GCG, ABEO and FIO managed the analyses of the study. Authors JFP and GO managed the literature searches are the project leaders. All authors read and approved the final manuscript.

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### ABSTRACT

**Introduction:** Carcinomas of the upper aerodigestive tract have a tropism on the epidermoid mucosa. HPV infection remains one of the risk factors for this cancer. This work aimed to study the presence of human papillomavirus (HPV) in carcinomas of the VADS.

**Materials and Methods:** This was a cross-sectional descriptive and analytical study with retrospective data collection over 7 years. The molecular analysis was conducted in Pointe-Noire using Xpert® HPV technology (GeneXpert, Cepheid). The variables studied were anatomopathological and virological.

\*Corresponding author: Email: [anicetboumba1974@gmail.com](mailto:anicetboumba1974@gmail.com);

**Results:** The overall molecular prevalence of oncogenic HPV was 28.1%. HPV-16 and HPV-18/45 were the incriminating genotypes in 88.9% and 11.4% of cases, respectively. A statistically significant association was found between co-infection with HPV-oncogenes in subjects over 40 years of age ( $P=0.01$ ) and the development of these HPVs in squamous cell carcinomas ( $p=0.02$ ). Notably, oncogenic HPV was found in the majority of laryngeal carcinomas.

**Conclusion:** In countries with limited resources, the Xpert HPV technology from GeneXpert is a reliable and rapid solution for the virological diagnosis of oncogenic HPV associated with carcinomas of the VADS. HPV-16 remains the most prevalent genotype.

*Keywords: Carcinoma; VADS; Xpert HPV; Géné Xpert; Congo.*

## 1. INTRODUCTION

Carcinomas of the nasopharyngeal cavity are epithelial malignancies that develop in the mucosa of the nasal-sinus cavity, pharynx, oral cavity, cervical esophagus, larynx and cervical trachea [1].

They represent 90% of all cancers of the ORL sphere and are responsible for 650,000 new cases per year in the world [1]. Although alcohol and tobacco intoxication is the main risk factor, many authors report the involvement of HPV in the carcinogenesis of ORL cancers [2,3].

Papillomaviruses are double-stranded DNA viruses belonging to the family Papillomaviridae that selectively infect squamous epitheliums and have a sexual route of transmission [3,4].

According to the World Health Organization (WHO), 660 million people worldwide are infected with high-risk oncogenic HPV that are believed to be involved in the development of squamous epithelial lesions at the level of VADS [5,6]. Unlike cervical cancer, HPV with oncogenic risk are involved in only 25% of pharyngolaryngeal cancers and in less than 50% of tonsil cancers for which the HPV-16 genotype is responsible in almost 90% of cases [7]. Several PCR techniques are being developed, but the GeneXpert technique makes it possible to simplify onco-virological diagnosis with a rapid rendering time of about 60 minutes for the detection of the 14 oncogenic high-risk HPV genotypes grouped in pool [8].

In Congo – Brazzaville Ondzotto et al. [7] conducted a study on the molecular diagnosis of oncogenic HPV on carcinomas of the larynx exclusively by the GeneXpert technique.

In view of the lack of data on cancer of the upper aerodigestive tract in Congo, this study was undertaken with the aim of identifying the

different genotypes of HPV in VADS cancers and establishing the correlation between HPV genotypes with patients' pathologic characteristics. The knowledge of the impact of HPV in VADS in Congo reflects the introduction of anti-infectious agents, especially anti-viral, in the chemotherapy of these cancers.

## 2. MATERIALS AND METHODS

We undertook a cross-sectional descriptive and analytical study. The data were collected retrospectively in the period from January 1, 2013 to December 31, 2019, i.e. 7 years.

This study was carried out in the oto-rhino laryngology (ORL) service of the Adolphe SICE Hospital in Pointe-Noire, the cytology and pathology anatomy laboratory of the Brazzaville Hospital and University Centre (CHU-B) and the Molecular Biology Laboratory of the Marie GOMBEZ Foundation in Pointe-Noire for molecular analyses.

The study population consists of the paraffin blocks of patients operated on in the different hospitals for cancer of the upper aerodigestive tract (VADS).

The inclusion criteria were all VADS cancers on the laboratory register thus allowing virological analysis for the detection of human papillomavirus (HPV) and amplification of oncogenic genotypes.

Poorly preserved or insufficient samples were excluded.

Out of a total of 52 blocks of VADS carcinoma tissue identified in the registries, we collected 32 cases thus constituting the size of our sample according to the inclusion criteria.

The equipment used consists of Eppendorfs tubes, sterile gloves, microtome blades, pliers,

tips, microtome, centrifuge, GeneXpert plc, oven, pipettes etc.

The study methods were carried out in three phases: the census, the collection of data and the technical management of the samples. The census phase consisted of searching the biotheque for cases of tissues corresponding to the carcinomas of VADS included in paraffin. The data collection phase was carried out in a dual epidemiological and biological investigation. The technical management of the samples was done by following the protocol of treatment of the specimens included in paraffin block.

Cuts of 5 µm were made at the microtome and then stored in eppendorfs tubes of 1.5 µL. After dewaxing with xylene and absolute alcohol, the samples were washed with PBS twice before being dried. The DNA extraction was carried out using the "ReliaPrep™ gDNA Tissue Miniprep System (Promega)" kit. Xpert®HPV technology (GeneXpert, Cepheid, USA) was used to amplify HPV DNA by real-time PCR, thus allowing the identification of genotypes.

The GeneXpert automated system enables both detection and identification of oncogenic HPV genotypes in a pool.

For the performance of the test, 1mL of re-suspended DNA was introduced into the Xpert HPV cartridge in the sample compartment. The cartridge was then introduced into the device and the test was launched. One hour later, the results of genotyping interpreted by the Xpert software were obtained:

- Either a positive result for HPV-16 Alone.
- Either a positive result for HPV 18/45
- Either a positive result for other high-risk HPV other than HPV 16 and / or HPV 18/45.
- A negative result for all high-risk HPV.

The variables studied were pathological (histological type) and virological (HPV-DNA, HPV type identified). The data was analyzed with STATISTIC SPSS 20.0 software. The chi-squared test was used to compare the results and a value of  $P < 0.05$  was considered significant between two variables.

### 3. RESULTS

#### 3.1 Pathological Characteristics Studied

Table 1 represents the histological types found in this study. Just over 78% of the types were squamous cell carcinomas, or 25 cases.

#### 3.2 Prevalence of High-Risk HPV

Table 2 represents the molecular prevalence of HPV-HR in VADS carcinomas. The molecular signature of oncogenic HPV was found in 28.1% of cases.

#### 3.3 HPV-HR Genotypes

The HPV-HR genotypes identified were represented in Table 3. The HPV-16 genotype was the most prevalent with 88.9% of positive cases.

#### 3.4 Bivariate Analysis

##### - HPV carrier association with histological type, cancer site and age

Tables 4-6 represents respectively the bivariate analysis between the HPV identified and the histological type, the site of the carcinomas and the age in our study. All HPV positive were identified in laryngeal squamous cell carcinomas ( $p=0.02$ ) and all patients were over 40 years of age ( $p=0.01$ ).

**Table 1. Histological types of VADS**

Histological types	Effectives (n)	Percentages (%)
Squamous cell carcinoma	25	78,1
Undifferentiated carcinoma of the nasopharynx or UNCT	5	15,6
Adenocarcinoma	2	6,3
Total	32	100

**Table 2. prevalence of HPV-HR in VADS**

HPV-HR	Effectives (n)	Percentages (%)
Positive	9	28,1
Negative	23	71,9
Total	32	100

**Table 3. HPV-HR genotypes**

Genotypes	Effectives (n)	Percentages (%)
HPV-16	8	88,9
HPV-18/45	1	11,1
Total	9	100

**Table 4. HPV association and Histological type**

Histological Type	HPV		Total
	HPV + n (%)	HPV- n (%)	
Squamous cell carcinoma	9(28,1)	16(50)	25(78,1)
Adenocarcinoma	0	2(6,3)	2(6,3)
Undifferentiated carcinoma or UNCT	0	5(15,6)	5(15,6)
Total	9(28,1)	23(71,9)	32(100)

**Table 5. HPV genotypes and the site of the carcinomas**

Siège	Génotype HPV		Total
	HPV 16	HPV 18/45	
Sinus	0	0	0
Cavum	0	0	0
Larynx	6(66,7)	1(11,1)	7(77,8)
Cavité buccale	2(22,2)	0	2(22,2)
Total	8(88,9)	1(11,1)	9(100)

**Table 6. Oncogenic HPV association and Age**

Age	HPV prevalence		Total
	HPV+ n(%)	HPV- n(%)	
< 20	0 (0,0)	2 (6,2)	2
20-40	0 (0,0)	7 (21,9)	7
> 40	9 (28,1)	14 (43,8)	23
Total	9	23	32

#### 4. DISCUSSION

Papillomaviruses are responsible for several cases of cancer worldwide. Since diagnosis is molecular, Xpert®HPV technology is one of the simplest and most reliable techniques for the identification of oncogenic HPV. This work aimed to study the presence of human papillomavirus (HPV) in VADS carcinomas among Congolese women.

Cepheid's Xpress HPV test has been used for the identification and genotyping of oncogenic HPV. It is a screening and genotyping method

that identifies 14 types of HPV with high oncogenic risk by using real-time PCR.

The results obtained in these studies showed a perfect agreement with other conventional techniques such as conventional PCR using the universal primers MY09/11 and GP5+/6+ [9, 10,11]. These results also showed that the Xpert-HPV system is a good molecular diagnostic method for HPV in paraffin-included biopsies [9, 10,11].

The overall molecular prevalence of HPV in VADS carcinomas was 28.1%. In the USA the

annual incidence of HPV+ VADS cancers is 0.65%, while the incidence of those related to alcohol and tobacco has been falling by 2.42% every year since 1983 [12]. DYYANI et al. (2016) report a prevalence of 36% of oncogenic HPV associated with carcinomas of the oropharynx [12].

The HPV16 genotype was the most common in our study with tropism in the larynx as confirmed by ZANG et al. (2017) in CHINA [10].

HPV types 16 and 18/45 are found respectively in the order of 88.9% and 11.1% in Congo, North America, KREIMER et al. (2018) SI MOHAMED et al. (2017) report respectively the majority genotype 16 in VADS carcinomas followed by genotype 18/45 [13,14]. Other high-risk genotypes are found in samples [14,15].

The laryngeal site is found in several works in the literature in the first rank of VADS carcinomas, it shares the same type of epithelium as the oropharynx justifying the participation of HPV in the mechanisms of carcinogenesis [16-20].

## 5. CONCLUSION

Virological diagnosis of high-risk oncogenic HPV associated with VADS carcinomas is made easy by GeneXpert technology. More than 28% of VADS carcinomas had an oncogenic HPV molecular signature. The larynx was exclusively infected with the HPV-16 genotype. Larger study would be needed to confirm this important molecular signature of HPV in VADS in Congo.

## DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

## ETHICAL APPROVAL

This work received the approval of the ethics committee for health research of the Marie Madeleine GOMBES Foundation under n° : 004/202 / CERS / FMMG-PN.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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