

Molecular signature of human papillomavirus in upper aerodigestive tract cancers in Congo-Brazzaville

Abstract

Introduction

Carcinomas of the upper aerodigestive tract (UADT) have a tropism on the epidermoid mucosa. The aim of this work was to study the presence of human papillomavirus (HPV) in carcinomas of the VADS.

Material 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.

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 ($p=0.02$). It is noted that oncogenic HPV were found in majority on 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.

Key words: Carcinoma, VADS, Xpert HPV, GeneXpert, Congo.

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 (6,8). Unlike cervical cancer, HPV with high 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 (13).

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.

The absence of data on the entire mucosa of VADS, allowed us to undertake this study with the aim of : identifying the different genotypes of HPV in VADS cancers.

Correlate HPV genotypes with patients' pathologic characteristics.

Material and Method

It was a cross-sectional and analytical descriptive 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 ENT department of the Adolphe SICE Hospital in Pointe-Noire, in the cytology and pathology anatomy laboratory of the Brazzaville Hospital and University Centre (CHUB) and in the Molecular Biology Laboratory of the Marie GOMBEZ Foundation in Pointe-Noire for molecular analyses.

The study population consisted 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 phase of technical management of the samples or procedure corresponds to a succession of treatment on paraffin block in a meticulous and codified way.

Cuts of 5µm were made at the microtome and then stored in eppendorfs tubes of 1.5 .mu.l. 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 plc makes it possible to carry out both the detection and the identification of oncogenic **HPV genotypes in 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.

Results

Pathological characteristics studied

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

Prevalence of high-risk HPV

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

HPV-HR genotypes

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

Bivariate analysis

- HPV association and Histological type

Tables IV and V represents the bivariate analysis between the HPV identified and age and the histological type 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 ($p=0.01$).

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. **The aim of this study was to study the molecular signature of oncogenic HPV in vads carcinomas in Congo.**

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+ (10, 11,12). These results also showed that the Xpert-HPV system is a good molecular diagnostic method for HPV in paraffin-included biopsies (10, 11,12).

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 (15). DYYANI et al. (2016) report a prevalence of 36% of oncogenic HPV associated with carcinomas of the oropharynx (15).

The HPV16 genotype was the most common in our study with tropism in the larynx as confirmed by ZANG et al. (2017) in CHINA(11).

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 genotype18/45 (16, 17).

Other high-risk genotypes are found in samples (17,18).

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 (20). ??

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

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Table I: 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 II: prevalence of HPV-HR in VADS.

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

Table III: HPV-HR genotypes

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

Table IV: 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 V: 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