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SCAN ME

Research Paper

MOLECULAR CHARACTERIZATION OF ONCOGENIC HPVS BY GENEXPERTIN CONGOLESE WOMEN IN THE CITIES OF POINTE-NOIRE AND DOLISIE

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Abstract

The classification of human papillomavirus (HPV) is based on their oncogenic power: non-oncogenic or low-risk HPVs and oncogenic or high-risk HPVs. However, studies on the molecular characterization of oncogenic HPVs remain less documented in Congolese women. The objective of this work was to characterize oncogenic HPVs in precancerous and cancerous lesions of the cervix in the cities of Pointe-Noire and Dolisie. The study involved 130 women who had received a vaginal swab from the cervix. The collected cells were placed using a cytobrush in SurePath ™ collectors. The sample was used for cytology and molecular analysis by real-time PCR. Microsoft Excel version 2013 and Graphpad Prism version 5.03 were used for statistical analyzes. Our results showed that the mean age of the women was 43.74 ± 10.30 years with extremities of 22 and 80 years. 75.40% at normal cytology; ASCUS at 3.10%; 3.10% low grade intraepithelial lesions (LSIL); 4.60% high grade intraepithelial lesions (HSIL) and 13.80% invasive carcinoma (CHF). Women The prevalence of High Risk Papillomaviruses (HPV-HR) was 41.50% (54/130). Overall, this study showed that 16.9% of women with normal cytology carried high-risk HPVs. In HSIL and ICC, 100% of these lesions consisted of high-risk HPV. Ultimately, HPV16 was the oncogenic genotype most implicated in HPV infections in the cities of Pointe-Noire and Dolisie.

INTRODUCTION

Cervical cancer remains a major health problem around the world, and mainly in emerging countries. Despite undeniable progress in the early diagnosis and prevention of this cancer, it is the 4th cancer in women in the world [1].

In 2012, an estimated 528,000 new cases of cervical cancer and 266,000 deaths from cervical cancer were estimated, 70% of which were in developing countries [2]. In sub-Saharan Africa, cervical cancer accounts for 22.5% of all cancer cases in women [3]. It is the leading cause of death and death in Africa behind breast cancer [2]. Congo has a population of 2,532,150 women, or 49.9% in 2019 [4].

Cervical cancer is the second most common female cancer in Congolese women and the second most common cancer in women aged 15 to 44 [5]. The age-adjusted incidence and death rates are estimated at 25.2 and 13.0 per 100,000 women, respectively [5].

Comprehensive data on genotyping, prevalence and distribution pose many challenges in Congo, on the association with risk factors associated with human papillomavirus (HPV) infection and cytology. The use of Pap smear Pap smear screening, molecular biology, and prophylactic HPV vaccines can effectively prevent cervical cancer in most cases. However, little data exists regarding the distribution of HPV genotypes in women in developing countries with a high incidence of cervical cancer.For Congo Brazzaville, data on HPV is only available in part of the republic where access is easy to care and to the technical platform of anatomy pathology and molecular biology. Thus, having precise data on the molecular characterization of HPVs could help prevent precancerous and cancerous lesions of the cervix in order to better determine the effectiveness of screening programs and treatments. The objective of this study was to establish the distribution of HPV16, HPV18, HPV45 and other HPVs most oncogenic by Genexpert in precancerous and cancerous lesions of the cervix in Congolese women.

MATERIALS AND METHODS

Type and Study Population

This was a descriptive, cross-sectional study that took place over a 22-month period from March 2017 until December 2018.

The women included in this study were recruited from the general hospitals of Dolisie and Pointe Noire. A total of 130 women who received a cervico-vaginal smear.

The examination was performed either at the request of the patient or by a health worker following any gynecological problem. Data was collected from a previously established survey sheet and after informed consent accepted by the patient. The study population consisted of all unvaccinated women over 18 years of age, known to have cancer, pregnant, and had a hysterectomy. Women who were physically or mentally unable to undergo an interview or cytology were not included.

Collecting cervical cells

During a cervical speculum examination, samples were collected using a cytobrush from the cervix. After scraping, cells were suspended in special vial collection vials containing

10 mL of BD SurePath ™ solution and stored at -20 ° C. The sample was used for cytology and molecular analysis.

HPV detection and genotyping

All HPV tests were performed on cervical cell samples stored in SurePath liquid medium at the Loandjili Hospital Laboratory in Pointe Noire. PCR was performed using the Genexpert-Cepheid system using the "Xpert-HPV" kit. The system allows the performance of real-time PCR in a flexible manner. Cepheids allow the identification of high risk oncogenic genotypes only. HPV 16, HPV 18/45 and other oncogenic HPVs are detected by this system. Extraction, amplification and detection are automated in 60 minutes.

Ethics

The study protocol was conducted in accordance with the principles of the Statement of the Ethics Committee for Research in Health Sciences (C.R.S.SA). All subjects gave their written informed consent for a biological assessment.

Statistical analysis

The development of figures was carried out using Microsoft Excel version 2013 software and the creation of graphs using Graph pad software version 5.03 (USA). The quantitative variables were expressed as means ± standard deviations. The threshold of $p < 0.05$ was used for statistical significance.

RESULTS

Sociodemographic characteristics with HPV groups

Sociodemographic characteristics with the HPV groups under study are presented below (Table 1). The mean age of the women was 43.74 ± 10.30 years, and that is 75% of the participants, were between 22 and 80 years old. DNA-HPV analysis was performed on 130 samples; HPV positive: 41.50% [age: 38.01 ± 10.83 years] and HPV negative: 58.50% [age: 46.2 ± 14.23 years] with an overall p value of age: $p < 0.0001$.

HPV genotyping

In total, 54 women were declared positive for one or more types of HPV-HR, ie a prevalence of 41.50% on 130 samples and 50.80% had a normal genotype (Table 2). The prevalence of Pointe-Noire is 29.2% (38/43) and the prevalence of Dolisie is 12.3% (16/33). The number of multiple infection types ranged from two to more than three HPV strains. The prevalence specific to each genotype is shown in Table 2 below.

Table 1: Sociodemographic characteristics according to HPV

Variables	Total		HPV négative		HPV positive	
	n	**%**	n	%	n	%
Ages (year)						
< 30	16	12.3	10	7.7	6	4.6
30 - 40	25	19.2	9	6.9	16	12.3
40 - 50	36	27.7	11	7.7	25	19.2
> 50	53	40.8	46	35.4	7	5.4
Age of first sexual intercourse (year)						
< 18	47	36.2	32	24.6	15	11.5
≥ 18	83	63.8	44	33.8	39	30
Number of sexual partners						
1	6	4.6	1	0.7	5	3.8
2	31	23.8	16	12.3	15	11.5
≥ 3	93	71.6	59	45.4	34	26.1
Number of pregnancies						
Nul	3	2.3	2	1.5	1	0.7
<5	41	31.5	25	19.2	16	12.3
≥5	86	66.2	49	37.7	37	28.5
Level of education						
Primary	37	28.5	28	21.5	9	6.9
Secondary	69	53.1	36	27.7	33	25.4
University	24	18.4	12	9.2	12	9.2
Marital status						
Single	72	55.4	48	36.9	24	18.5
Married	53	40.8	27	20.8	26	20
Divorced / Widowed	5	3.8	1	0.8	4	3.1
Smoking status						
No	121	93.1	74	56.9	47	36.1
Yes	9	6.9	2	1.5	7	5.4
Alcoholic Status						
No	63	48.5	35	26.9	28	21.5
Yes	67	51.5	41	31.6	26	20
Use of oral contraception						
No	74	56.9	52	40	22	16.9
Yes	56	43.1	24	18.5	32	24.6

Table 2: Prevalence of HPV types by city

HPV Types		Pointe-Noire		Dolisie	
	n	n	%	n	%
HPV -	**76**	**43**	**33,1**	**33**	**25,4**
HPV +	**54**	**38**	**29,2**	**16**	**12,3**
16	23	16	12,3	7	5,4
18/45	3	3	2,3	0	0
16/18/45	2	1	0,8	1	0,8
16/Others	8	6	4,6	2	1,5
Others	**18**	**12**	**9,2**	**6**	**4,6**

Features of genotyping with cytology

This study shows that 50.8% of women with normal cytology were negative for HPV infection; 3.1% (ASCUS); 3.1% (LSIL); 4.6% (HSIL) and 13.8% (ICC) (Table 2). In HSIL and CHF, 100% of these lesions consisted of high-risk HPV. HPV16 is the most oncogenic genotype implicated in HPV infections and cervical cancer in our study.

Table 3: Genotype characteristic according to cytology

HPV Types			normal		ASCUS		LSIL		HSIL		ICC	
	n	%	n	%	n	%	n	%	N	%	n	%
HPV -	**76**	**58.5**	**66**	**50.8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
HPV +	**54**	**41.5**	**22**	**16.9**	**4**	**3.1**	**4**	**3.1**	**6**	**4.6**	**18**	13.8
16	23	17.7	13	10	2	1.5	1	0.8	2	1.5	4	3.1
18/45	3	2.3	2	1.5	0	0	0	0	0	0	1	0.8
16/18/45	2	1.6	0	0	0	0	0	0	1	0.8	1	0.8
16/others	8	6.1	5	3.8	0	0	0	0	2	1.5	1	0.8
Others	**18**	**13.8**	**2**	**1.5**	**2**	**1.5**	**3**	**2.3**	**1**	**0.8**	**11**	**8.5**

ASCUS: Squamous cell atypia of undetermined significance. **LSIL**: Low-grade squamous intraepithelial lesion. **HSIL**: High-grade squamous intraepithelial lesion. **ICC**: invasive cervical carcinoma.

DISCUSSION

Epidemiological data on the genotyping of HPV strains and on cervical cancer are limited in the Republic of Congo. Thus, the objective of this work was to establish a distribution of oncogenic HPVs using the Genexpert system in precancerous and cancerous lesions of the cervix.

This study was carried out on 130 women whose average age was 43.74 ± 10.30 years, to draw its interest on the knowledge of the genotypes of HPV16, HPV18, HPV45 and other oncogenic HPVs in various precancerous and cancerous cervical lesions of the cervix. All age-specific studies on HPV have shown that young women are the most vulnerable to this infection, which is acquired during the first sexual intercourse [6].

The results of this study showed an overall prevalence of HPV infection at 41.5%. This prevalence was found to be practically equal to the prevalence of BOUMBA [7] which was 41.1%. Likewise in Gordana Kovacevic in Serbia [8] with a prevalence of 51.8%, compared to the prevalence observed in the majority of European studies are between 11.3% and 18.3%. The difference in prevalence may be a result of geographic areas, choice of HPV types and risk factors [9, 10].

The significant prevalence of HPV infection found in this study with a p: <0001 could be explained by the absence of screening programs organized in the country, the lack of information and awareness of the population on this pathology. , the limited number of pathology and molecular biology laboratories, but also the high population density in this area and unsafe sexual practices. These factors mean that women who consult are potentially at risk because they only consult after a gynecological problem.

This study focused on the most oncogenic high-risk genotypes of which HPV 16 (17.70%) and other oncogenic HPV (13.80%) were the most frequent. These results are in agreement with studies carried out by BOUMBA et al. on the distribution and of which the genotypes HPV16 (20.6%) and 33 (14.3%) were the most encountered in Congo [7]. However, this work is different from the literature where the HPV16 and 18 genotypes are the most oncogenic [11].

In this study, the distribution of these genotypes based on cytologic diagnosis showed 50.80% of women were negative for HPV infection. Women with an ASCUC cytology diagnosis had 3.1% infection due to high-risk genotypes. Although the world average in this category is 10.4% [12] and 12.3% in South Saharan Africa [13]. In this category of women, HPV 16 was the most prevalent genotype.

Exclusively oncogenic genotypes or high risk HPVs have been observed in low grade, high grade lesions and invasive cancer. These results agree with most studies in the world [14].

CONCLUSION

In short, we have established a genotypic profile of the most oncogenic HPV genotypes circulating in southwestern Congo in women with normal cytology, in different grades of precancerous and cancerous lesions of the cervix. HPV 16 is the oncogenic genotype most implicated in HPV infections and cervical cancer in Congo.

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