ISSN: 03875547
Volume 45, Issue 10, December, 2022

Epidemiological and clinical profile of Human Papillomavirus infection associated with cervical cancer in Brazzaville

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Keywords:
Cervical cancer, HPV infection, Epidemiological, clinical,

ABSTRACT

Human papillomavirus infection is associated with cervical cancer, one of the major public health problems in developing countries. In the Republic of Congo, despite the high incidence rate, estimated at 25.5% among women, molecular epidemiology data on HPV infections are still limited. We studied the prevalence of HPV in cervical smears of patients who came for screening during a cervical cancer screening campaign. Liquid-based cytology samples were collected for cytologic diagnosis and HPV detection. Nested PCR was performed using HPV consensus primers). Of the 131 women examined, 41 (31,3%) cases of normal cytology and 56 (42,7%) cases of benign cellular changes (BCM) were diagnosed, 18 (13,7%) cases of undetermined cellular atypia (ASCUS), 8 (3,8%) cases of low-grade intraepithelial lesions (LSIL), 5 (2,3%) cases of high-grade intraepithelial lesions (HSIL), and 3 (3%) cases of invasive cancers (ICC). Our results showed that 70/131 (53%) of total samples were positive for HPV DNA. In women without lesions, an age-specific prevalence of HPV was observed in all age groups. The main local risk factors for HPV infection in women with lesion-free and lesioned cytology were: age, risky sexual behavior, multiple sexual partners, age of first sexual intercourse. In conclusion, could be used as an evidence base for future epidemiological surveillance, emphasizing that in addition to Pap smears, HPV testing should be considered in cervical cancer screening and diagnosis to provide a significant opportunity for national health programs to control cervical cancer and save women's lives.

1. INTRODUCTION

Genital human papillomavirus (HPV) infection is the most common sexually transmitted infection. It is the main etiological agent for the development of cervical cancer [1- 4]. The transmission rate of HPV during sexual intercourse is 5-100%; 60% of women would be infected within 5 years of their first intercourse. The diagnosis of HPV infections is primarily clinical. They are most often asymptomatic. The clinical examination should be complemented by a Pap smear, a sexually transmitted infection test and colposcopy. The treatment of human papillomavirus genital lesions is not perfectly codified, which explains the large number of methods used. Prevention remains the best weapon to defeat HPV infection. While persistent infection with high-risk HPV is the primary risk factor for developing cervical cancer, other host or environmental factors may influence the risk of HPV infection progressing to malignancy. Several other factors have been found to increase the risk of cervical cancer, possibly through their relationship to the risk of HPV infection: number of sexual partners, early sexual activity, parity, long-term oral contraceptive use, smoking, and HIV/AIDS [5].

Cervical cancer (CC) is a major cause of morbidity and early death worldwide with an incidence of 604,127 new cases (13.3%), it represents about 10% of all cancers affecting more specifically low-resource countries like Africa [6] Indeed, CC is the leading cause of death in women from cancer, 214 deaths (14.2%) from cervical cancer occur and are diagnosed each year in the Republic of Congo [7]. In recent years (2015-2022), a few small-scale studies have reported the prevalence of HR-HPV associated with CC in different departments of Congo with incidences 41.1%, 37.5%, 5.38% respectively 8-10. These high incidences of HPV in the population is a reflection of a lack of awareness among Congolese youth and also a lack of UCC screening [8], [10].

CC is one of the few preventable cancers, so its prevention relies on early examination of cancerous lesions and a focus on cell diagnosis during Pap smears. Differences between low- and high-income countries were related to differences in exposure to risk factors and adequacy of screening. Lack of smear screening and HPV testing in women on a regular basis is a major risk factor for increased cervical cancer cases. Two retrospective studies on the cytological screening history of patients treated for advanced CC showed that about 70% of them had never had a Pap smear or were only occasionally followed up [11], [12]. Currently, an unacceptable number of advanced cancers have been found in older women with Pap test, so we need to use other more sophisticated and effective methods to detect cancer at an early stage for better management. Our objective is to reveal the different epidemiological and clinical factors contributing to the persistence of HPV infection and to show the relationship between the detection of the virus and the data of the cytological examination as well as to verify if the molecular detection of this virus could contribute to improve the diagnosis of precancerous lesions of the uterine cervix.

2. Material and methods

2.1 Study population

This descriptive cross-sectional study was conducted in January 2022 in the gynecology departments of two (2) health facilities: the "Le Fort" clinic and the Brazzaville University Hospital Center (CHU-B). The patients who came for consultation were motivated by certain gynecological problems such as bleeding after sexual relations, pelvic pain, vaginal discharge, pruritus, exploration of infertility, and others for a routine check-up. After a routine gynecological examination and Pap smear prescription, 131 women underwent uterine cervical Pap smear.

Eligible patients included all women visiting gynecological services regardless of their medical history during

the recruitment period. All patients were also subjected to a standardized questionnaire regarding their lifestyle and sexual activity. Excluded from the study were pregnant women, menstruating women, women undergoing known treatment for cervical lesions, women who were physically or mentally unable to undergo an interview or cytological examination, and those who did not provide informed consent. The study was approved by the Congolese Ethics Committee for Health Sciences Research.

2.2 Data and sample collection

For each patient, a survey questionnaire was completed with data on sociodemographic characteristics, including age, age of first sexual intercourse and function, information on alcohol and tobacco consumption, number of pregnancies, as well as information on sexual behavior (number of multiple partners, contraceptive use, risky sexual behaviors (cunnilingus, fellatio, and sodomy), history of sexually transmitted infections (STIs)) were collected. Cervical samples were stored in special tubes containing 4 mL of PreservCyt® Transport medium and stored at -80 ◦C at the Laboratory of the University Hospital of Brazzaville. Transport of samples for molecular study was performed under dry ice following the recommendations of good laboratory practices (GLP) at the Laboratory of Virology, Oncology, Biosciences, Environment and New Energy (LVO BEEN) at the Faculty of Sciences and Techniques of Mohammedia, Hassan II University of Casablanca in Morocco.

2.3 Cytological diagnosis

After sampling, the samples were sent to the pathology laboratory of CHU-B for cytological study by a pathologist. The results were reported according to the Bethesda 2001 11 classification system.

2.4 HPV molecular assay

2.4.1 DNA extraction

DNA extraction from cervical samples was performed using the One-4-All Genomic DNA Mini-prep kit according to the manufacturer's instructions. The eluate was stored at -80 ◦C until further use.

2.5 Evaluation of the DNA extract

The extracted DNA was assayed in NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE). Quality and absence of PCR inhibitors were analyzed by amplifying a 268-bp beta-globin household gene using GH20/PC04 primers as described by [13].

2.6 Molecular detection of HPV

HPV-DNA detection was performed by nested PCR using the consensus primers MY09/MY11 and GP5+/GP6+ of 450 bp and 150 bp respectively in the highly conserved region of the L1 gene 13.

For the detection of Betaglobin and HPV, PCRs were performed in a total volume of 25µL of reaction mixture, including 12.5µL master mix Green Taq Mix, 2µL of sense and antisense primers each, 2 µL of DNA template, and 6.5 µL of distilled water. The PCR product from the first PCR (external reaction) was then used as a template for the nested-PCR (internal reaction) under the same conditions.

The PCR amplification program for beta-globin was as follows: initial denaturation at 94 ºC for 5 min; followed by 30 cycles of denaturation at 94 ºC 40 s, hybridization at 61 ºC 30 s, extension at 72 ºC, 1 min and final extension at 72 ºC for 5 min. HPV amplification consisted of an initial denaturation step at 94 ºC for 5 min, then a denaturation step at 94°C for 30 sec followed by hybridization for 1min at 55˚C or 48˚C extension at 72°C for 1min and finally a final extension step at 72˚C/ 7min. DNA from the Hela cell line (one to two viral copies of DNA/cell HPV18) was used as a positive control and ultrapure PCR water (Bioline, UK) as a

negative control to assess the success of amplification. The products of all PCRs were analyzed by 2% agarose gel electrophoresis (Promega)

2.7 Statistical analysis

Socioepidemiological data entry was performed using Excel 2016 software. IBM SPSS Statistic 22 software was used to perform non-parametric tests, Mann-Whitney U tests for quantitative data, Fisher exact t or Chi2 square tests for qualitative data and $p<0.05$ was considered significant.

3. Results

All enrolled participants had a mean age of 42.7±10 years with extremes ranging from 21 to 71 years with a peak between the age range of 41 and 50 years. We studied some risk factors related to HPV infection in women associated with cervical cancer including: age, number of pregnancies, number of sexual partners, age of first sexual intercourse, risky sexual behavior, contraceptive use, sexually transmitted infections (Table 1). The most represented women had had sexual intercourse before the age of 18 (61.8%), 58.7% had had less than 5 lifetime sexual partners and the highest number of pregnancies was among women with more than 5 pregnancies (45.03%). In our study population, many women did not use contraceptives (53.4%), there was a high alcohol consumption (54.2%), sexual risk behaviors were high (54.9%), few had already contracted a sexually transmitted disease (17.5%). The women who frequented gynecology services the most were Congolese state employees (18.3%).

Table 1: Distribution of socio-demographic characteristics

Age range	**Frequency**	**Percentage**
≥21-30	35	26,7
31-40	23	17,5
41-50	43	**32,8**
≥51	30	22,9
Age of first sexual intercourse		
< à 18	81	**61,8**
≥ à 18	50	38,1
Multiple sexual partners		
< 5	77	**58,7**
≥ 5	54	41,2
Past STI		
Yes	108	82,4
No	23	17,5
Risky sexual behaviour		
Yes	72	**54,9**
No	59	45,03
Number of pregnancies		
0	30	22,9
≥5	59	**45,03**
<5	42	32,06
Contraceptive use		
Yes	61	46,5
No	70	**53,4**

ISSN: 03875547
Volume 45, Issue 10, December, 2022

Alcohol consumption			
Yes		71	**54,2**
Non		60	45,8
Function			
Administrative officer		11	8
Health Officer		19	14,504
Other jobs		19	14,504
Clerk		15	11,450
Teacher	6		4,580
Student		12	9,160
Congolese state employee		**24**	**18,321**
Retired	7		5,344
Unemployed		18	13,740

3.1 Clinical profile

More women came to the gynecology department with symptoms of vaginal discharge and/or vaginal pruritus (40%) followed by pelvic pain (Table2).

Table 2: Distribution of the clinical profile

Clinical profile	Frequency	Percentage
Routine check-up	15	11%
Pelvic pain	40	31%
Exploration of infertility	13	10%
Vaginal discharge and/or pruritus	**52**	**40%**
Bleeding after sexual intercourse	11	8%

3.2 Cytological profile

Of the 131 women included, 41 (31.3%) cases of normal cytology and 56 (42.7%) cases of benign cellular changes (BCM) were diagnosed, 18 (13.7%) cases of undetermined cellular atypia (ASCUS), 8 (6.1%) cases of low-grade intraepithelial lesions (LSIL), 5 (3.8%) cases of high-grade intraepithelial lesions (HSIL), and 3 (2.3%) cases of invasive cancer (ICC) (Table 2).

Table 3: Distribution of cytological profile in Congolese women

Cytology		Frequency	Percentage
ASCUS		18	13,7
HSIL	5		3,8
ICC	3		2,3
LSIL	8		6,1
Benign cellular modification		**56**	**42,7**
Normal		41	31,3

3.3 Prevalence of HPV DNA

HPV DNA detection was performed in cervical samples from 130 Congolese women attending gynecology clinics. Our results showed that 46/70 (66%) of the total samples were positive for HPV DNA (Figure 1).

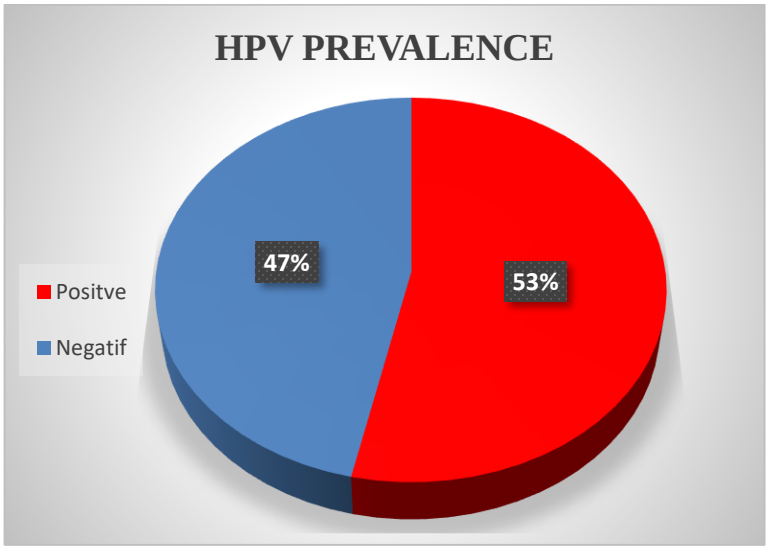

Figure 1: HPV infection in Congolese women

3.4 HPV infection and sociodemographic characteristics by severity of cervical lesions

The sociodemographic and sexual characteristics of the women studied according to the severity of cervical lesions and HPV infection are summarized in (Table 4). Statistical analysis in women with normal cytology was evaluated to identify some risk factors. The main local risk factors for HPV infection in women with lesion-free and lesioned cytology were: age (p=0.01), risky sexual behavior (p=0.02), multiple sexual partners (p=0.03), age of first sexual intercourse (0,001) and no significance was found with contraceptive use and number of pregnancies (p>0.05).

Table 4: Distribution of socio-demographic characteristics of women by severity of cervical lesions and HPV infection

Characteristics	Abnormal		Normal		p value
	HPV -	HPV +	HPV -	HPV +	
Tranche d'âge	**%**	**%**	**%**	**%**	
≥21-30	7,14%	10,00%	19,15%	22,00%	**0,01**
31-40	28,57%	20,00%	38,30%	28,00%	
41-50	28,57%	30,00%	14,89%	**32,00%**	
>51	35,71%	**40,00%**	27,66%	18,00%	
Multiple sexual partners					
<5	57,14%	**65%**	46,81%	**68%**	**0,03**
≥5	42,86%	35%	53,19%	32%	
Number of pregnancies					
Aucun	0,00%	14,29%	27,27%	20%	0,4
≥5	50,00%	**62,86%**	46,36%	**53,33%**	
<5	50,00%	32,86%	36,36%	26,67%	
Risky sexual behavior					
Yes	28,57%)	**65%**	54,32%	**58%**	**0,02**
No	71,41%	35%	44,68%	42%	

Age of first sexual intercourse					
<18	64.29%	60%	61,70%	**62%**	
≥18	35,71%	40%	38,30%)	38%	**0,001**
Use contraceptive					
Oui	57,14%	**65%**	55,32%	**58%**	0,7
Non	42,86%	35%	44,68%	42%)	

p<0.05 with age, sexual partners, age of first sexual intercourse, risky sexual behavior

4. Discussion

Worldwide, HPV testing for cervical cancer-associated HPV DNA is accepted as a reliable and valid option for the management of women with equivocal cytologic findings. And in recent years, there has been increasing interest in using HPV testing on cervical samples from asymptomatic women without cytologic abnormalities [14]. This strategy will allow for early and effective management of populations at different risk levels for this neoplasia because of the close relationship between HPV infection and the development of cervical cancer. The Republic of Congo does not have an organized national UCC screening program. Few efforts have been made to reduce the prevalence of UCC by organizing screening campaigns. To date, epidemiological data on HPV prevalence and genotypes are fragmentary. My work was guided by this perspective, with the aim of determining the prevalence of HPV infection in Brazzaville based on the cytological status of women attending the gynecology department and also to highlight factors that may be associated with cervical cancer and HPV in order to raise awareness of the population to protect themselves.

Of 131 cervical specimens collected according to the inclusion criteria, 97 were confirmed as having lesion-free cervical cytology and were included, representing 90% of the baseline sample. The mean age of the women in this study was 42.8± 10 years with extremes ranging from 21 to 71 years with a peak between 31 and 40 years. Our results are slightly similar to those of [10], [9], [5] who reported mean ages of 43.74± 10.30 years, 43.67± 12.31 years, 43.0 years± 12.8 years respectively 9,10,15. This observation could be explained by the fact that the mean age of the population has not changed in recent years in studies on HPV and CRC in Congolese women.

The cytological profile was 31.3% normal cytology; 42.7% MCB, 13.7% ASCUS and 2.9% LSIL 6.1%, 3.8% HSIL and 2.8% CCU. Our results are much lower than those of [15]. who found in their study a rate of 87% for normal cytology, 30.8% for ASCUS, 61.5% LSIL, 7.7% HSIL and 1.8% of UCC in Gabon. This difference can be explained by the sample size of our study. Furthermore, our results are very similar to those of Samira Zoa Assoumou in that our higher percentages are found in normal cytologies than in abnormal ones. UCC being the 2nd most common cancer among Congolese women, only 25.9% of our study population had a cytological abnormality. This relatively low rate shows how much the Congolese population does not undergo routine screening, in this case older women, due to lack of information and awareness of the disease, and it will present itself at an advanced stage where the disease can no longer be treated. Because the young population with HPV could spontaneously eliminate the virus in case of infection by their immune system unlike the elderly [16].

Our results showed a high prevalence of HPV infection (53%). Comparable rates were also recorded in Burkina faso (66.1%), Gabon (60%), Guinea Conakry (50.8%) and South Africa 48.1% [16- 19]. This prevalence may be explained by the lack of a UCC screening campaign and national vaccination program in our country. Prevalence rates of HPV infection ranging from 13% to 40% have been reported in low-risk or

general populations in sub-Saharan African countries [20- 25]. The 53% prevalence of HPV found in the present study, which is much higher than the 40% prevalence found in similar high- risk populations in Africa [26], [27], may reflect the high sexual exposure of our study population and places our country as endemic.

Overall, the prevalence of HPV infection was present in all cytologic profiles studied, with a peak on abnormal cytologies (40%). These results are alarming compared to the reported global prevalence of HPV in women of 10.4% [28]. In Africa, similar studies report lower prevalence, including Morocco (15.8%) [29] and Benin (26.7%) [30]. Variations between studies most likely reflect differences in the study population, as well as their lifestyle that may expose them to HPV more quickly. The presence of HPV infection in all smears with abnormal cytology highlights the close causality of HPV in precancerous lesions and ICCs, i.e., the prevalence of HPV increased with the severity of cervical lesions, this HPV infection remains dormant at undetectable levels at first contact in youth and reactivates thereafter. The possibility that older women may experience reactivation of latent HPV infections [31], [32]. No significant relationship was found ($p>0.05$).

Some risk factors have been identified as increasing the risk of HPV infection and acting in conjunction with it to induce cervical cancer, such as age, parity, oral contraception, smoking, age of first sexual intercourse, marital status, and history of sexually transmitted infections (STIs) such as Chlamydia trachomatis and HI [33]. In our study, a statistically significant association was found between HPV infection and age, risky sexual behavior, multiple sexual partners, first sexual intercourse. These observations are consistent with the various risk factors reported in the literature [34]. This finding underscores that more risk factors related to sexual components are associated with HPV infection and may therefore reflect the sexual behavior and depravity of morals in Congolese youth. It is worth noting that women attending the gynecology department present more for reasons of pelvic pain and pruritus and/or discharge, although these factors are not counted as risk factors, they could be indicator factors to alert us to take a consultation and then be screened for precancerous lesions by PAP tests and HPV tests.

Our results also show the interest of UCC screening for a better follow-up of HPV infection in women diagnosed without cervical lesions in our country.

5. Conclusion

Our study confirms the permanent presence of HPV in the Congolese population. They also show the interest of doing routine controls at one's gynecologist. This study is still ongoing in order to increase the sample size and the PCR products have been sent for sequencing. Given the lack of awareness of HPV infection and cervical cancer screening in women with both abnormal and normal cytology, the health system should improve conditions for better management of cervical cancer in the Republic of Congo.

Acknowledgements

The authors thank the management of the Hospital and University Hospital of Brazzaville (CHU-B), Congo for facilitating the transport of the samples to Morocco as well as the Laboratory of Virology, Oncology, Biosciences, Environment and New Energies, Faculty of Science and Technology of Mohammedia, Hassan II University of Casablanca, Morocco. We are grateful with a special memory to all the women who participated in this study.

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