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Prevalence and Genotypic Distribution of Human Papilloma Virus (HPV) in Precancerous Lesions of the Cervix in Brazzaville, Republic of the Congo

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Abstract

Background: Human papillomavirus (HPV) is the primary etiological agent of cervical cancer. In the Republic of Congo, data on the distribution of high-risk HPV (HR-HPV) genotypes remain limited. This study aimed to determine the prevalence and distribution of HR-HPV genotypes in archived precancerous cervical lesion samples in Brazzaville. **Methods:** A retrospective study was conducted on 83 formalin-fixed paraffin-embedded (FFPE) cervical biopsies collected between 2020 and 2022. Following DNA extraction, HR-HPV detection and genotyping were performed using multiplex qPCR targeting HPV16, HPV18, and a pool of 12 other oncogenic genotypes. Statistical significance was assessed using Fisher's exact test and continuity-corrected odds ratios (OR). **Results:** Successful gene amplification was achieved in 69.9% (58/83) of samples. The overall HR-HPV prevalence was 55.4% (46/83) for the entire cohort and 79.3% (46/58) among samples with confirmed DNA quality. HPV16 was the most frequent genotype (78.3%), followed by HPV18 (17.4%). A significant correlation was found between HPV16 and lesion severity ($p = 0.006$), with 100% of high-grade squamous intraepithelial lesions (HSIL) testing positive for HPV16. In contrast, HPV18 and other genotypes were exclu-

sively detected in low-grade lesions (LSIL). **Conclusion:** These findings demonstrate a high prevalence of HPV16 in precancerous lesions in Brazzaville, reinforcing its critical role in local cervical carcinogenesis. Current vaccination strategies targeting types 16 and 18 appear highly relevant for the Congolese context.

Keywords

High-Risk HPV, Precancerous Lesions, Cervix Uteri, qPCR, Brazzaville, Congo, Genotyping

1. Introduction

Cervical cancer is a malignant epithelial neoplasm whose etiology is intrinsically linked to persistent infection with DNA viruses of the Papillomaviridae family: Human Papillomaviruses (HPV). This pathology results from uncontrolled cell proliferation in the transformation zone of the cervix, evolving from asymptomatic precancerous lesions to an invasive carcinoma capable of metastasizing [1] [2].

Globally, cervical cancer remains the fourth most diagnosed cancer in women, with 604,127 new cases and 341,831 deaths annually (GLOBOCAN 2020) [3]. Sub-Saharan Africa bears the brunt of the burden, with cervical cancer leading the way in female cancer mortality, accounting for 15.5% of deaths [4]. In the Republic of Congo, although it is the second most common cancer after breast cancer, it is the leading cause of cancer death in women [5].

The central role of HPV in cervical carcinogenesis is now a scientific certainty, with viral DNA detected in more than 99% of squamous cell carcinoma cases [6]. Among the hundred or so identified genotypes, approximately thirteen are classified as high-risk oncogenic (HR-HPV): types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 [1]. These strains possess transforming potential linked to the expression of E6 and E7 oncoproteins, which interfere with the host's cellular regulatory cycles.

The fight against this cancer relies on primary prevention through vaccination and secondary prevention through screening and treatment of precancerous lesions. The effectiveness of these strategies, particularly vaccination, depends on a precise understanding of the local epidemiology of oncogenic HPV genotypes, whose geographical distribution varies considerably. While current vaccines primarily target HPV types 16 and 18 (predominant in Europe), their effectiveness is closely linked to the local prevalence of circulating strains. However, the virological mapping of Congo remains incomplete making it difficult to assess the impact of available vaccines.

In Congo, studies conducted in the departments of Pointe-Noire, Bouenza and Niari have begun to draw a partial map of HPV-HR [7]-[10]. To date, no data have been produced on the molecular characterization of HPV in precancerous lesions in Brazzaville. This gap is all the more critical given that management is

often hampered by socioeconomic factors and late diagnoses [11]. Understanding the local virological dynamics is therefore essential to refining screening and vaccination strategies.

Our study investigates the correlation between viral strains found in invasive cancers and those circulating in precancerous stages. We hypothesize that the genotypic profile and molecular prevalence of HPV in precancerous lesions are similar to those observed in cancerous forms.

To verify this hypothesis, we set ourselves the general objective of characterizing HPV strains in precancerous lesions of the cervix at the University Hospital of Brazzaville.

2. Material and Methods

2.1. Type, Framework and Period of Study

We conducted a cross-sectional analytical study with retrospective data collection over a seven-month period (May to November 2022). The work was carried out at two complementary sites in the Republic of Congo:

- **The Laboratory of Anatomical and Cytological Pathology at the University Hospital of Brazzaville:** for cohort selection, microtomy and histological confirmation.
- **The Hugues Dieudonné Loemba (HDL) Molecular Biology Laboratory of the MM Gombes Foundation (Pointe-Noire):** for the critical steps of dewaxing, DNA extraction and HPV genotyping.

2.2. Biological Material and Sampling

The study material consisted of paraffin-embedded cervical biopsies (FFPE - Formalin-Fixed Paraffin-Embedded) archived between January 2020 and June 2022. Out of a total of 126 identified cases, 83 blocks were selected according to the following criteria:

- **Inclusion:** Histological diagnosis confirmed of "precancerous lesion".
- **Exclusion:** Insufficient tissue for molecular analysis, absence of exocervical lining on review, or unusable blocks after double-blind histological review (WHO classification 2014).

2.3. Histological Analysis

Sections of 3 to 5 µm thickness were prepared using a microtome (LEICA). Standard hematoxylin-eosin (H&E) staining was performed to confirm the grade of precancerous lesions prior to molecular analysis. The slides were mounted under synthetic resin (Eukitt) and validated by a pathologist.

2.4. Molecular Virology Procedures

2.4.1. Pretreatment and Dewaxing

For each sample, 5 to 10 µm sections were collected in sterile tubes. Dewaxing was performed using a thermal and chemical method:

1) Heating at 90˚C for 5 minutes in molecular biology water, followed by decantation (repeated twice).

2) Washing with 70% ethanol and centrifugation at 6000 rpm for 5 minutes.

3) Drying of the pellet at 37˚C and final washing with phosphate saline buffer (PBS).

2.4.2. Extraction of Genomic and Viral DNA

Extraction was performed using the Canvax Genomic DNA Tissue Kit. The critical phase of enzymatic lysis was optimized by digestion with Proteinase K (20 mg/ml) incubated for 24 hours at 60˚C in a water bath, ensuring the release of paraffin-trapped DNA. After enzyme inactivation at 95˚C, the DNA was eluted in 50 µL of EB buffer and stored at −80˚C.

2.4.3. DNA Quantification

The DNA concentration of each extract was normalized using the Qubit 3.0 fluorometer (Life Technologies). This nucleic acid-specific fluorescence technology allowed for adjusting the DNA load to optimize PCR sensitivity.

2.4.4. HPV Genotyping by Multiplex qPCR

Amplification and genotyping were performed on a magnetic induction thermocycler (MIC qPCR Cycler) using the High-risk HPV DNA Diagnostic Kit (Sansure Biotech). This TaqMan® multiplex system allows for the simultaneous detection of high-risk oncogenic HPV genotypes (HR-HPV) on four fluorescence channels:

- **ROX Channel:** Specific detection of HPV16.
- **VIC Channel:** Specific detection of HPV18.
- **Canal FAM:** Pooled detection of 12 other HR genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
- **Channel Cy5:** Endogenous internal control (beta-globin) to validate extraction and the absence of inhibitors. In other words, serving as a control of the integrity of the DNA extracted from the paraffin blocks (often degraded by formalin).

The protocol included polymerase activation (95˚C, 2 min), followed by 40 cycles of denaturation (95˚C, 5 s) and hybridization/elongation (60˚C, 30 s). The sensitivity of the test is 400 copies/ml.

Software: The analysis of the fluorescence curves was carried out using BMS (Biomolecular Systems) software version 2.10.

Appendix A: Optimization of the reaction mixture (Master Mix)

The final reaction volume was set at 20 µL. The mixture was prepared under sterile conditions in a laminar flow hood to avoid any contamination by amplicons (Table 1).

Appendix B: Thermal cycler protocol (MIC qPCR)

The amplification program was configured to optimize polymerase kinetics and the specificity of TaqMan probe hybridization (Table 2).

Sample suitability criteria for Qpcr

Suitability criteria: DNA concentration ≥10 ng/µL and DO260/280 ratio be-

tween 1.8 and 2.0.

Table 1. Optimization of the reaction mixture (Master Mix).

Components	Volume per reaction (µL)
qPCR master mix (2X)	10 µL
Primer and probe mixture (Multiplex HPV-HR)	1 µL
RNase/DNase-free water	4 µL
Master mix volume	**15 µL**
DNA extracted (sample or control)	5 µL
Final total volume	**20 µL**

Table 2. Thermal cycler protocol (MIC qPCR).

Stage	Temperature	Duration	Cycles
Polymerase activation	95˚C	2 min	1 (Hold)
Initial denaturation	95˚C	5 min	1 (Hold)
Denaturation	95˚C	5 seconds	40
Hybridization & elongation*	60˚C	30 seconds	

*Fluorescence reading (FAM, VIC, ROX and Cy5 channels) is performed at the end of each elongation step.

2.4.5. Statistical Analyses

The data was entered into Microsoft Excel 2019 and analyzed with RStudio (v4.2). Qualitative variables are expressed as percentages. Comparisons were performed using Pearson's chi-squared test or Fisher's exact test, with a significance threshold set at $p < 0.05$. Association strengths were estimated by calculating odds ratios (OR) with their 95% confidence intervals.

2.4.6. Ethical Considerations

The study received approval from the Research Ethics Committee for Health Sciences (CERSSA). Anonymity and data confidentiality were strictly maintained. In accordance with best practice guidelines, the blocks were returned to the tumor bank at the University Hospital of Brazzaville (CHU-B) after analysis.

3. Results

3.1. Sociodemographic Characteristics of the Study Population

The study included a sample of N = 83 patients. The median age was 43 years (range: 22 to 82 years), with a marked predominance of women over 40 years of age (59%). Geographically, the majority of samples came from Brazzaville (85.5%), with the remainder distributed between Pointe-Noire, Nkayi, and Dolisie (Table 3).

Table 3. Sociodemographic distribution of the cohort (N = 83).

Variables	Number (N)	Percentage (%)
Age range (years)		
≤30	12	14.5
31 - 40	22	26.5
> 40	49	59.0
Origin		
Brazzaville	71	85.5
Pointe-Noire	6	7.3
Nkayi	3	3.6
Dolisie	3	3.6

3.2. Histological Profile of Precancerous Lesions

Anatomopathological analysis, performed according to the 2014 WHO classification, revealed a predominance of low-grade squamous intraepithelial lesions (LSIL), identified in 69.9% of cases (N = 58). High-grade lesions (HSIL) were present in 30.1% of the sample (N = 25), among which CIN2 stages were the most common (25.3% of the total cohort) (Table 4).

Table 4. Histological characteristics of the samples (N = 83).

Histological grade	Number (N)	Percentage (%)
Low grade (LSIL)	**58**	**69.9**
High grade (HSIL)	**25**	**30.1**
Including CIN2 (Moderate)	21	25.3
Including CIN3 (Severe)	4	4.8

3.3. Performance of Extraction and Amplification by qPCR

Of the 83 samples tested, qPCR amplification was successful for 58 samples (69.9%) (Table 5). The 25 non-amplified samples (30.1%) were distributed as follows: 18 failed the internal control (β-globin gene) and 7 showed amplification of the internal control but no amplification of the HPV targets.

3.4. Molecular Prevalence and Genotyping of High-Risk Human Papillomavirus (HR-HPV)

3.4.1. Prevalence of High-Risk Human Papillomavirus (HPV)

Of the 58 samples successfully amplified, 46 were positive for at least one high-risk HPV genotype (HR-HPV), representing a prevalence of 79.3% (95% CI: 66.6 - 88.1).

Considering the entire initial cohort (N = 83), the overall prevalence of HR-

HPV was 55.4% (46/83).

3.4.2. Distribution of Genotypes

Molecular typing of the 46 HPV-HR positive samples revealed a clear predominance of HPV16, identified in 78.3% of cases (N = 36). HPV18 was present in 17.4% of cases (N = 8). The overall prevalence of HR-HPV across the entire study population (N = 83) was found to be 55.4% (46/83) (Table 5). A pool of 12 other high-risk genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) was detected in 4.3% of cases (N = 2). No co-infections were observed in this series.

Table 5. Results of HPV-HR detection and genotyping.

Molecular parameters	Number (n)	Percentage (%)
Sample suitability (N = 83)		
Successful amplification (suitable DNA)	**58**	**69.9**
Amplification failed (DNA degraded)	25	30.1
HPV status (N = 58)		
Positive (HR-HPV)	**46**	**79.3**
Negative	12	20.7
HPV status (among total cohort, N = 83)		
Positive (HR-HPV)	46	**55.4**
Unamplified/Negative	37	44.6
Distribution of genotypes (N = 46)		
HPV16	**36**	**78.3**
HPV18	8	17.4
Other HPV-HR (Pool) *	2	4.3

**Pool of 12 genotypes: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68*.

3.4.3. Regional Genotypic Mapping

Comparison of our results with previous data shows a disparity in HPV prevalence in southern Congo. While HPV16 remains the dominant genotype throughout the country, the highest prevalence is observed in Pointe-Noire (81.5%), closely followed by Brazzaville (79.3%; N = 58), while the departments of Bouenza and Niari show lower rates (<40%).

3.5. Bivariate Analyses: Associated Factors and Correlations

3.5.1. Correlation between Age and Histological Grade

Analysis of the distribution of lesion grades by age shows that HSILs are proportionally stable across age groups, although the raw number is higher in women over 40 years of age due to the cohort structure. No statistically significant association was established between age and lesion severity (p = 0.985) (Table 6).

Table 6. Association between age groups and histological types.

Age range (years)	LSIL n(%)	HSIL n(%)	OR [IC 95%]	p-value
≤30	8 (13.8)	4 (16.0)	0.8 [0.2 - 3.1]	0.793
31 - 40	16 (27.6)	6 (24.0)	1.2 [0.4 - 3.5]	0.734
>40	34 (58.6)	15 (60.0)	0.9 [0.4 - 2.5]	0.907

3.5.2. Association between HPV Genotypes and Histological Grade

A highly significant association was observed between certain genotypes and histolo-gical severity. HPV16 showed a major correlation with high-grade lesions, being present in 100% (17/17) of genotyped HSILs, compared to 65.5% of LSILs ($p = 0.006$). Conversely, HPV18 and other types were identified only in low-grade lesions within this sample.

Table 7. Distribution of HPV genotypes according to histological grade (N = 46).

Genotypes	LSIL N = 29 (%)	HSIL N = 17 (%)	OR [IC 95%]**	p-value*
HPV16	19 (65.5)	17 (100)	18.30 [1.02 - 328.20]	**0.006**
HPV18	8 (27.6)	0 (0, 0)	0.00 [0.00 - 0.86]	**0.017**
Other HPV-HR	2 (6.9)	0 (0, 0)	0.00 [0.00 - 6.10]	0.523

*P-value calculated by Fisher's Exact Test (recommended for sample sizes <5). **OR calculated with Haldane-Anscombe continuity correction for null cells.

4. Discussion

Our study, conducted on a cohort of 83 women in Congo, aimed to characterize the epidemiological and virological profile of precancerous cervical lesions. The findings highlight key aspects regarding the prevalence of high-risk human papillomavirus (HR-HPV), the distribution of genotypes, and their association with lesion severity, in line with evidence reported in the international literature.

4.1. Methodological and Virological Considerations

The use of formalin-fixed paraffin-embedded (FFPE) cervical biopsies as biological material is a cornerstone of retrospective molecular epidemiology. Although formalin fixation ensures excellent preservation of tissue architecture for histology, it induces chemical cross-links between proteins and nucleic acids, which can fragment viral DNA. This limitation explains our amplification rate of 69.8% (58/83). The observed amplification failures are likely due to suboptimal viral load or DNA degradation related to overfixation, a phenomenon documented by Steinau *et al*., who emphasize the importance of the pre-analytical phase in molecular virology [12].

The choice of thermal dewaxing with a Poupinel lamp (90˚C) proved strategic. Unlike chemical dewaxing with solvents (xylene), heat promotes the breaking of formalin-DNA bonds, thus improving primer accessibility during PCR. The real-

time PCR (PCR) technique used here offers superior sensitivity and analytical specificity compared to conventional PCR, enabling accurate detection even in the presence of low copies of the HPV viral genome [1] [7] [10]. Indeed, Steinau *et al*. in 2011 compared two dewaxing methods and obtained better results with the heat dewaxing technique than with the xylene-based technique [12].

4.2. Virological Analysis of Prevalence and Genomic Distribution

4.2.1. High Prevalence of High-Risk HPV (HR-HPV)

The overall HR-HPV prevalence of 55.4% (reaching 79.3% among evaluable samples) is high, yet remains consistent with data reported for populations pre-selected on the basis of histological abnormalities.

Although the amplification rate of 69.9% was limited by DNA degradation inherent to formalin-fixed paraffin-embedded (FFPE) blocks, the prevalence of 79.3% observed among analyzable samples highlights the pervasive presence of high-risk HPV in the precancerous lesions studied. This finding aligns with the international scientific consensus attributing nearly 100% of cervical carcinomas to these oncogenic viruses [13] [14]. The molecular prevalence identified at Brazzaville University Hospital is comparable to the 81.5% previously reported in Pointe-Noire [7], reinforcing the concept of a substantial viral burden in cervical pathogenesis in Congo. To mitigate the impact of these infections, often exacerbated by limited access to screening [11], the use of HPV testing—particularly self-sampling—appears to be a priority strategy to optimize management in fragile health system settings [15] [16].

4.2.2. Distribution of Genotypes and Associated Risks

HPV amplification was only possible on 58 of the 83 extracted samples. Of these 58 amplified samples, 46 were high-risk (HR) HPV, representing an HPV prevalence of 79.3%. Of the 46 positive cases, 36 were genotyped as HPV16 (78.3%), the most prevalent genotype. The remaining genotypic profiles consisted of HPV18 (17.4%) and other HR-HPV types, which accounted for only 4.3% of cases.

Our prevalence is close to that of Boumba *et al*., (2014) in Pointe-Noire, who reported 81.5% in HSIL [7]. But it does not corroborate with Siriaunkgul *et al*., (2008) in Thailand and Tsimba Lemba *et al*., (2022) in Congo, who reported a prevalence of 96.9% and 100% respectively in confirmed cancer cases [9] [17].

This prevalence is higher than that reported in studies by Veríssimo Fernandes J *et al*. (2011) in Brazil, Belglaiaa (2016) in France, Kande (2018) in Burkina Faso, and Mutombo *et al*. (2019) in the DRC, which reported prevalences of 24.5%, 23.7%, 16.7%, and 28.2%, respectively [18]-[21]. These variations could be explained by the nature of the study population. These authors worked with cervical cytology cases, so their study population also included normal cytology samples, which significantly reduces the prevalence of HPV.

Our genotypic results corroborate those of Pretet *et al*. (2008) in France, showing a predominance of HPV16 (73%), followed by HPV18 (19%) and other high-risk HPV types [22]. In Mozambique, a clear predominance of HPV16 and 18 was

also noted [23]. In Sudan, Abate *et al*. (2013) found similar results, with 82.5% HPV16, followed by other high-risk HPV types [24]. Boumba *et al*. (2014) in southwestern Congo noted some discrepancies with our results [7]. While they did report a predominance of HPV16 at 47.1%, HPV18, the second most frequently identified genotype in our series, was preceded in their study by HPV33 (22.6%). HPV18 was the third most frequently identified genotype, in 15% of cases. Methodological differences between our studies could explain these observations. Indeed, we used real-time PCR for HPV genotype detection. Boumba *et al*. used conventional PCR and sequencing for HPV genotype detection [7].

The distribution of genotypes in our study is striking due to the hegemony of HPV16, found in 78.3% of positive cases. This result is consistent with the literature, which places HPV16 as the most oncogenic and most frequent type in precancerous lesions and invasive cancers [13] [14].

One particularly interesting finding concerns HPV18. In our study, it was detected only in low-grade lesions (27.6% of LSILs) and was absent from HSILs. Although surprising at first glance, this result is consistent with a growing body of evidence showing that, in cross-sectional screening studies, the immediate risk of CIN2+/CIN3+ associated with HPV18 may be lower than for other types such as HPV 16, 33, or 31 [25] [26]. It is ranked lower in terms of PPV for precancerous lesions [25]-[27]. However, as studies on invasive cancers remind us, HPV18 remains the second most common type after HPV16 [13] [14]. This discrepancy is explained by the fact that HPV18 is less efficient at producing detectable high-grade precancerous lesions, but has the potential to progress rapidly to cancer, sometimes escaping screening. This observation reinforces the idea that genotyping alone, particularly for HPV18, may not be sufficient for triage due to its lower sensitivity compared to cytology [28]. He emphasizes the need for careful monitoring, even in the absence of immediate high-grade lesions.

4.3. Regional Genomic Mapping and Technological Biases in Congo

The median age of our cohort (43 years) is consistent with the African literature (Boumba *et al*., Mutombo *et al*.). From a virological perspective, this data is crucial: it corresponds to the period of clinical latency after the initial HPV infection (generally contracted at the beginning of sexual activity). The delay of approximately 10 to 15 years between persistent infection and the appearance of high-grade lesions (HSIL) explains why our population is younger than that of patients with invasive carcinomas reported by Tsimba Lemba *et al*. (54.5 years) [9].

The distribution of the study population according to histological types showed that low-grade squamous intraepithelial lesions (LSIL) were the most represented, accounting for 69.9% of cases. We obtained moderate high-grade squamous intraepithelial lesions (HSIL) in 25.3% and severe high-grade HSIL in 4.8%. Belglaiaa (2016) in France also found a predominant frequency of LSIL [14]. Konaté *et al*. (2019) in Mali reported a predominance of moderate HSIL, representing 42.2% of cases in the entire population, which consisted of precancerous and cancerous lesions of the cervix [1].

The predominance of LSILs (69.9%) reflects the early stage of virus-induced genomic instability. In these lesions, the viral genome is generally maintained in episomal form, while the transition to HSILs is often marked by the integration of the HPV genome (particularly type 16) into the host genome, leading to overexpression of E6 and E7 oncoproteins.

The epidemiology of high-risk human papillomaviruses (HR-HPV) in the Republic of Congo is currently documented by several studies conducted in the southern departments of the country. This genotypic mapping is based on data collected in distinct geographical areas, revealing significant disparities.

In Pointe-Noire, the work of Boumba *et al*., focusing on high-grade intraepithelial lesions (HSIL), revealed a very high prevalence of 81.5% [7]. Concurrently, in the Bouenza and Niari departments, Nganga *et al*. reported significantly lower rates, at 39.2% and 37.5%, respectively [10]. Our own work conducted in Brazzaville corroborates the observations from Pointe-Noire, with a prevalence of 79.3% among patients with HSIL.

Comparative analysis highlights a major geographical contrast: while Brazzaville and Pointe-Noire show comparable and high prevalence rates (approximately 80%), Bouenza and Niari have rates half as high (approximately 38%). This discrepancy is likely methodological. The study by Nganga *et al*. [10] was based on cervical smears from a population in which 86% of subjects had normal cytology. The large-scale inclusion of women without cervical lesions mechanically reduces the overall HPV prevalence, unlike the Brazzaville and Pointe-Noire studies, which specifically targeted populations with confirmed precancerous lesions.

From a virological standpoint, HPV16 predominates across all studies, generally followed by types 33 and 18. However, the frequency of genotypes after HPV16 varies between departments, a heterogeneity that could result from the different PCR platforms used. Nganga *et al*. employed GeneXpert® technology, which groups results into pools (HPV16, HPV18/45, and "other HPV-HR"), thus limiting the individual distinction of genotypes within the pools [10]. Conversely, the conventional multiplex PCR used by Boumba *et al*. in Pointe-Noire, as well as the specific genotyping method used in our study in Brazzaville, allows for individualized and more precise identification of each viral type [7] [10]. These technological nuances would explain the variations in prevalence observed for genotypes such as HPV33 and HPV18 depending on the study area.

4.4. Analysis of the Epidemiological and Histological Profile

The median age of our cohort (43 years) is consistent with the African literature (Boumba *et al*., Mutombo *et al*.). From a virological perspective, this data is crucial: it corresponds to the period of clinical latency after the initial HPV infection (generally contracted at the beginning of sexual activity). The delay of approximately 10 to 15 years between persistent infection and the appearance of high-grade lesions (HSIL) explains why our population is younger than that of patients

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4.5. Viro-Histological Correlation and Oncogenic Risk

The lack of a significant association between age and histological grade ($p = 0.987$) suggests that the risk of progression depends less on biological age than on the duration of viral persistence and the specific type of HPV.

Bivariate analysis highlighted a highly significant association between HPV16 and high-grade lesions (HSIL), where it was present in 100% of cases. This translates into a very high odds ratio (OR = 18.30; $p = 0.006$) (**Table 7**). This result perfectly corroborates the data in the literature which classify HPV16 as the genotype with the highest positive predictive value (PPV) for CIN2+ and CIN3+ [25] [27]. Our study thus confirms that, in our context, the presence of HPV16 should be considered a major warning sign justifying rapid management, ideally by immediate colposcopy, as suggested by genotype-based triage strategies [29].

The fact that HPV16 is ubiquitous in HSIL reinforces the need for molecular screening specifically targeting this genotype to identify women at the highest risk of progression to cancer. Conversely, the detection of HPV18 exclusively in LSIL suggests a different behavior, perhaps a less frequent progression towards a high grade, a phenomenon that would merit further investigation.

This result confirms the superior oncogenic potential of HPV16, which is capable of progressing more rapidly to severe dysplasia compared to other genotypes. The absence of type 18 and other high-risk HPV types in the HSILs of our sample (**Table 7**) could be related to our sample size after successful amplification, but it reinforces the idea that HPV16 is the main driver of cervical carcinogenesis in Brazzaville.

4.6. Role of Other Genotypes and Perspectives for Sorting

The use of a pooled multiplex kit for "other HPV-HR" in this study does not allow for precise identification of the respective share of genotypes 31, 33 or 45 in the remaining 4.3%.

Although this study identified only two cases (4.3%) of non-16/18 high-risk HPV (classified as "other"), these were associated only with LSIL. However, the literature emphasizes the importance of extensive genotyping. Types such as HPV 33, 31, and 58 are frequently associated with a high risk of CIN2+/CIN3+ [25] [30] [31]. Their presence, like that of HPV16, could justify faster referral for colposcopy in a personalized triage strategy [29] [32]. Conversely, so-called "intermediate risk" types such as HPV 39, 59, or 51 could allow for one-year follow-up while awaiting viral clearance, thus easing the pressure on healthcare systems [25] [33]. The absence of these genotypes in our HSIL series is probably related to the size of our sample, but does not call into question their importance in screening programs.

4.7. Implications for Vaccination Prevention

Our data on the distribution of genotypes in precancerous lesions have direct implications for the vaccination strategy in Brazzaville. The overwhelming predominance of HPV16, followed by HPV18, reinforces the usefulness of a vaccination program. Unlike some regions of Africa where types 35, 45 or 52 are frequently reported [1] [33], Brazzaville has a molecular profile that is very favorable to the effectiveness of first-generation vaccines. The quadrivalent vaccine (targeting HPV 6, 11, 16, and 18) would cover almost all the genotypes responsible for HSIL in our study. If our population had included more types such as 31, 33, 45, 52, and 58, as suggested by global studies [33] [34], the benefit of a nonavalent vaccine would be even more pronounced, potentially preventing more than 90% of cervical cancers linked to these genotypes [35]. These results provide an essential local epidemiological basis to help health authorities assess the potential impact and choose the most appropriate vaccine to implement as part of a national vaccination program. [36].

4.8. Limitations of the Study and Strengths of the Study

We must acknowledge several limitations to our work. The main one is the modest sample size and the rate of DNA degradation (30.1%), which limited the statistical power to analyze in detail the role of rare genotypes or co-infections. In addition, the cross-sectional nature of the study does not allow us to assess the progression or regression of lesions over time, a crucial aspect for understanding the natural history of infection by different genotypes [25]. Finally, the lack of concomitant cytological data did not allow us to evaluate the performance of genotyping as a triage test in combination with other markers.

However, this study has notable strengths. It relies on a gold-standard histological diagnosis for all patients, validated by experienced pathologists, ensuring the reliability of the lesion classification [37]. It provides valuable local data in the context of the Congo, where epidemiological data on the distribution of HPV genotypes in precancerous lesions are scarce. These results constitute an important factual basis for guiding screening and vaccination policies in the region.

4.9. Selection Bias and Confounding Factors

This study has certain limitations that should be highlighted for a cautious interpretation of the data:

- Selection bias: The cohort consists of patients who have already consulted at specialized facilities for suspicious lesions. This "selected" population does not necessarily reflect the prevalence of HPV in the general female population of Brazzaville.
- Missing clinical data: The use of archival data limited access to essential clinical covariates such as HIV serological status, smoking, parity, or age at first sexual intercourse. HIV, in particular, is a major confounding factor known to increase the persistence of HPV infection and promote multiple infections with less common genotypes. In the absence of this data, our results should be considered a basic virological characterization of the lesion rather than a multifactorial risk analysis.

5. Conclusion

This work establishes a solid foundation in molecular virology for the Republic of Congo. establishes that precancerous lesions of the cervix in Brazzaville are associated with a very high prevalence of infection by high-risk HPV, with a clear dominance of the HPV16 genotype, strongly linked to the most severe lesions. This molecular signature argues for an urgent strengthening of targeted vaccination and HPV testing in urban areas. These data suggest that vaccination coverage targeting types 16 and 18 could significantly reduce the incidence of cervical cancer in Congo. However, DNA degradation in archived tissues underscores the need to improve storage conditions for more effective genomic surveillance.

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Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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