Prevalence and Distribution of Human Papillomavirus Genotypes Among Women in Kinshasa, The Democratic Republic of the Congo

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abstract

PURPOSE Cervical cancer is the leading cause of mortality by cancer in sub-Saharan Africa. The human papillomavirus (HPV) infection is recognized as a necessary and sufficient cause for cervical cancer. Population-specific estimates of HPV prevalence in the Democratic Republic of the Congo (DRC) are unknown. This study aims to estimate the prevalence of HPV and identify predominant genotypes circulating in Kinshasa, DRC.

METHODS Between July 2015 and July 2017, women were invited to attend a screening program at Mont-Amba Health Centre in Kinshasa. Cervical specimens were collected using the Preservcyt medium. HPV DNA testing was performed for all specimens using real-time polymerase chain reaction.

RESULTS During the 2-year period, a total of 1,870 women age 25 to 82 years were screened. The mean age was 46 years (± 11.4 years). The overall HPV prevalence was 28.2% (95% CI, 26.1% to 30.3%). High-risk HPV prevalence was 24.8% (95% CI, 22.8% to 26.8%). Women younger than 30 years had the highest overall HPV prevalence (42.2%; 95% CI, 34.7% to 49.9%). A second peak of prevalence was observed in women age 60 years and older. HPV68 (5.5%; 95% CI, 4.5% to 6.6%) was the most prevalent HPV type.

CONCLUSION The distribution of HPV genotypes among women in our population was different compared with other world regions. A key finding was that HPV68 was the most prevalent high-risk HPV genotype. These findings highlight the need for the determination in our population of the etiologic fraction of different HPV types in invasive cervical cancers.

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INTRODUCTION

Cervical cancer is a major public health problem worldwide. Annually, there are 569,847 new cases, with an estimated 311,400 related deaths.1 It is the second most common cancer among women in low- and middle-income countries, especially in sub-Saharan Africa, which accounts for 83% of all new cases.2

Human papillomavirus (HPV) is the main causative agent of cervical cancer.3 To date, more than 150 HPV types have been identified. The International Agency for Research on cancer has categorized HPV types into high-risk (HrHPV) and low-risk (LrHPV) types according to their potential to induce malignancy. Fourteen HrHPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) were oncogenic and extensively studied for their role in cervical cancer.4-7 HPV infection is the most common sexually transmitted infection and is usually cleared by the host immune system within a few years after acquisition.8 Persistent infections with specific HPV genotypes can cause cellular changes that develop into cervical intraepithelial neoplasia and, eventually, into invasive cervical cancer.9 Worldwide, 10.4% of women with normal cervical cytologic findings are carrying HPV infection.10 Higher prevalence was found in less-developed regions: 22.1% in Africa and 20.4% in Central America and Mexico, compared with Northern America (11.3%), Europe (8.1%), and Asia (8.0%).11 A study published in 2010 on women with normal cytology showed the highest prevalence of HPV (23.2%) in women younger than 25 years of age. The prevalence dropped to 8.7% and 5% in women between 25 and 34 years and women older than 35 years, respectively.12

Globally, HPV16, HPV18, HPV33, HPV45, and HPV31 are the most prevalent HPV types involved in invasive cervical cancer.13 The relative importance of each HPV type may differ by region. HPV16 and HPV18 contribute to more than 70% of all cervical cancer cases.14 HPV DNA testing has become an acceptable alternative to cytology for an accurate diagnosis of patients...
The overall prevalence rate of HPV infection was 28.2% and particularly high among young women age 25 to 29 years. A second peak of prevalence was found in the group of women age 60 years and older. Through polymerase chain reaction, 18 HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68) have been identified. A key finding is that HPV68, which is not covered by current anti-HPV vaccines, was the most prevalent high-risk HPV genotype in our environment.

Relevance
This result highlights the need for the identification of HPV types involved in biopsy-proven cervical carcinomas. Future anti-HPV vaccines should expand coverage against additional HPV types, which will be found the most prevalent in invasive cervical cancers.

Collection of Cervical Samples
After providing informed consent to participate, all eligible women were subjected to gynecologic examination. While lying in the lithotomy position on the examination table, a trained health worker inserted a bivalve speculum inside the vagina to visualize the cervix. A cervix broom was then used to collect a cervical specimen for liquid-based cytology. The cervix specimen collected was transferred into a vial with the PreservCyt solution (Hologic, Marlborough, MA) and stored at room temperature between 15°C and 30°C, according to the manufacturer. All specimens collected were sent for analyses at Algemeen Medisch Laboratorium BVBA, in Antwerp, Belgium. Algemeen Medisch Laboratorium is Part of the National Reference Centre for HPV in Belgium.

HPV Testing and Genotyping
HPV testing was performed by using the Riatol quantitative polymerase chain reaction (PCR) HPV genotyping test. This is an International Organization for Standardization–certified, fully automated, clinically validated laboratory-developed PCR method for HPV genotyping. The laboratory was blinded from all clinical data. Processing of the samples was performed in batches of 91 samples. On arrival at the laboratory, cervical samples in PreservCyt solution were placed in the Sample Transfer System (Hologic), and representative aliquots of 2 mL were transferred in a deep-well plate. These 2-mL aliquots were placed in the Medium Throughput Automation (Hologic) for fully automated DNA extraction. Extraction was done exploiting standard boom extraction with magnetic beads using the Genfind DNA extraction kit (Hologic). Thereafter, sample DNA was amplified on the LightCycler 480 (Roche). The presence of different HPV genotypes was determined using a series of TaqMan-based real-time PCRs targeting type-specific sequences of viral genes.

CONTEXT
Key Objective
Is the type-specific prevalence of human papillomavirus (HPV) circulating in the general population in Kinshasa different from other world regions?

Knowledge Generated
The overall prevalence rate of HPV infection was 28.2% and particularly high among young women age 25 to 29 years. A second peak of prevalence was found in the group of women age 60 years and older. Through polymerase chain reaction, 18 HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68) have been identified. A key finding is that HPV68, which is not covered by current anti-HPV vaccines, was the most prevalent high-risk HPV genotype in our environment.

Relevance
This result highlights the need for the identification of HPV types involved in biopsy-proven cervical carcinomas. Future anti-HPV vaccines should expand coverage against additional HPV types, which will be found the most prevalent in invasive cervical cancers.
quantitative PCR HPV test not only detects 14 HrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) but also reports selected potential high-risk or low-risk HPV types (6, 11, 53, and 67). The PCR reactions were done in ultra-low volume (6 μL) and were performed in eight multiplex reactions. Cellularity control was performed on every sample, by amplification of the β-globin gene. On the basis of the β-globin standard curve, DNA concentration (nanograms per microliter) was determined in every sample. Samples with a DNA concentration below 0.12 ng/μL were considered invalid and reported as not evaluable.

The final categorical results were recorded as follows: HPV negative (no HPV detected), HrHPV positive (sample positive for at least one HrHPV type), LrHPV positive (sample negative for HrHPV but positive for at least one LrHPV), not evaluable (sample not evaluable because of insufficient cells/insufficient DNA concentration).

Statistical Analyses
HPV prevalence was calculated by the ratio of the number of HPV-positive women divided by the total number of women tested. Type-specific HPV prevalence was determined as the proportion of women positive for a given HPV genotype among all women tested. We calculated a 95% CI for each reported prevalence rate. All statistical analyses were performed using the STATA statistical analysis software packages (Version 15; Stata, College Station, TX).

Ethical Issues
This study was approved by the Ethical Committee of the University of Kinshasa School of Public Health. It was conducted following the Good Clinical Practice requirements and in accordance with the principles of the World Medical Association Declaration of Helsinki and subsequent relevant amendments.

Role of the Funding Source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS
Characteristics of the Study Population
During the 2-year period, a total of 1,870 women were enrolled in the study. These women were of different backgrounds and locations in the city, allowing a representability sufficient to extrapolate the results to the entire population. Participants' demographics, sexual history, and health history are summarized in Table 1.

The mean age was 46 years (± 11.4 years). According to age, the study population was divided into 5-year intervals for women age 20 to 29 years and into 10-year intervals for women age 30 years and older. The final age group

| Characteristics | Frequency (N = 1,870) | % |
|-----------------|----------------------|---|
| Age, years      |                      |   |
| 25-29           | 176                  | 9.4|
| 30-39           | 386                  | 20.6|
| 40-49           | 585                  | 31.2|
| 50-59           | 487                  | 26.0|
| ≥ 60            | 236                  | 12.6|
| Mean            | 46 ± 11.4            |   |
| Marital status  |                      |   |
| Single          | 289                  | 15.5|
| Married         | 1,199                | 64.1|
| Separated       | 153                  | 8.2|
| Widowed         | 229                  | 12.2|
| Education       |                      |   |
| None            | 108                  | 5.8|
| Primary         | 336                  | 18.0|
| Secondary       | 1,010                | 54.0|
| Superior        | 416                  | 22.2|
| Profession      |                      |   |
| None            | 709                  | 37.9|
| Remunerated     | 782                  | 41.8|
| Nonremunerated  | 379                  | 20.3|
| Gynecologic history |                |   |
| Menopausal status |                    |   |
| No              | 1,019                | 54.5|
| Yes             | 851                  | 45.5|
| Pregnancies     |                      |   |
| 0               | 120                  | 6.4|
| 1               | 165                  | 8.8|
| 2-4             | 437                  | 23.4|
| ≥ 5             | 1,148                | 61.4|
| Parity          |                      |   |
| 0               | 273                  | 14.6|
| 1               | 176                  | 9.4|
| 2-4             | 542                  | 29.0|
| ≥ 5             | 879                  | 47.0|
| Abortions       |                      |   |
| 0               | 1,065                | 57.0|
| 1               | 488                  | 26.1|
| ≥ 2             | 317                  | 17.0|
| Miscarriages    |                      |   |
| 0               | 1,128                | 60.3|
| 1               | 456                  | 24.4|
| ≥ 2             | 286                  | 15.3|

(Continued on following page)
TABLE 1. Characteristics of Participants Attending a Community-Based Cervical Cancer Screening Program in Kinshasa, Democratic Republic of the Congo (2015 to 2017) (Continued)

| Demographics                  | Frequency (N = 1,870) | %   |
|-------------------------------|-----------------------|-----|
| **Sexual history**            |                       |     |
| Age at first intercourse, years |                       |     |
| 10-19                         | 1,349                 | 72.1|
| 20-29                         | 495                   | 26.5|
| 30-39                         | 25                    | 1.3 |
| ≥ 40                          | 1                     | 0.1 |
| Mean                          | 18.2 ± 3.55           |     |
| **Lifetime sexual partners**  |                       |     |
| 1                             | 592                   | 31.7|
| 2-5                           | 1,103                 | 58.9|
| 6-10                          | 140                   | 7.5 |
| 11-19                         | 18                    | 1.0 |
| 20-29                         | 11                    | 0.6 |
| 30-39                         | 5                     | 0.2 |
| ≥ 40                          | 1                     | 0.1 |
| **Oral contraceptive use**    |                       |     |
| No                            | 1,476                 | 78.9|
| Yes                           | 394                   | 21.1|
| **Condom use**                |                       |     |
| No                            | 1,096                 | 58.6|
| Yes                           | 774                   | 41.4|
| **Use of traditional herbs inside the vagina** | | |
| No                            | 885                   | 47.3|
| Yes                           | 985                   | 52.7|

*Traditional substances (leaves and powders) that women insert into the vagina for supposed personal hygiene, disease prevention or treatment, and enhancement of sexual experience. These practices can cause damage to the vaginal epithelium or changes in vaginal flora. The consecutive genital irritations might facilitate the transmission of pathogenic organisms.

comprised women who were at least 60 years of age. A total of 176 women (9.4%) were younger than 30 years. Women age 40 to 49 years constituted the most populated age group (31.2%).

HPV Results

As detailed in Table 2, among the 1,870 samples collected from women at baseline, 521 samples (27.8%) tested positive for HPV, 1,325 samples (70.9%) had no virus identified on HPV DNA testing, and 24 samples (1.3%) were not evaluable because of insufficient DNA concentration. Out of 521 HPV-positive samples, 457 (24.4%) were HrHPV positive. Exclusive LrHPV infections were found in 64 samples (3.4%). Mixed infections (one or more HrHPV types combined with one or more LrHPV types) were found in 54 samples (2.9%). On cytology, we found low-grade squamous intraepithelial lesions in 24 HPV-positive samples, low-grade squamous intraepithelial lesions in 43 HPV-positive samples, and squamous cell carcinoma in only three HPV-positive samples.

Age Distribution of HPV Infections

Within the study population, which comprised 1,846 participants, the overall prevalence of HPV infections was 28.2% (95% CI, 26.1% to 30.3%). The prevalence rate of HrHPV was 24.8% (95% CI, 22.8% to 26.8%). Women age 25 to 29 years had the highest overall HPV prevalence at 42.2% (95% CI, 34.7% to 49.9%). Compared with their younger counterparts, the overall prevalence declined in women age 30 to 39 years (27.7%; 95% CI, 23.2% to 32.5%), 40 to 49 years (27.6%; 95% CI, 24.0% to 31.4%), and 50 to 59 years (23.8%; 95% CI, 20.0% to 27.8%). A second peak was observed in the group of women age 60 or more years (29.2%; 95% CI, 23.5% to 35.4%). Table 3 shows the overall prevalence of HPV infections per age group.

Type-Specific HPV Prevalence

In 1,846 women with valid HPV results, 126 LrHPV types and 653 HrHPV types were found. As reported in Table 4, HPV genotypes varied substantially within age groups. HPV68 was the most prevalent in all age groups (5.5%; 95% CI, 4.5% to 6.6%). The most prevalent LrHPV type was HPV53 (3.2%; 95% CI, 2.4% to 4.1%). Figure 1 shows the 10 most prevalent HPV types in descending order.

Proportion of HPV Types per Woman

A single HPV type was encountered in 366 (70.2%) HPV-positive women, and 155 (29.8%) were harboring multiple HPV types. As shown in Figure 2, the prevalence rates for single HPV infection and multiple HPV infection were 19.8% (95% CI, 18.0% to 21.6%) and 8.3% (95% CI, 7.0% to 9.6%), respectively. The average number of HPV types per woman was two (0 to 11).

DISCUSSION

In this study, we estimated the prevalence of HPV genotypes among women age 25 years and older in a community of Kinshasa in the DRC. This was one of the largest...
| HPV Result | 25-29 (n = 173) | 30-39 (n = 375) | 40-49 (n = 579) | 50-59 (n = 483) | ≥ 60 (n = 236) | Total (N = 1,846) |
|------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| HrHPV      | 65 (37.6 (30.3 to 45.2)) | 88 (23.5 (19.2 to 28.0)) | 142 (24.5 (21.0 to 28.2)) | 99 (20.5 (16.9 to 24.3)) | 63 (26.7 (21.1 to 32.8)) | 457 (24.8 (22.8 to 26.8)) |
| LiHPV      | 8 (4.6 (2.0 to 8.9)) | 16 (4.3 (2.4 to 6.8)) | 18 (3.1 (1.8 to 4.8)) | 16 (3.3 (1.9 to 5.3)) | 6 (2.5 (0.9 to 5.4)) | 64 (3.4 (2.7 to 4.4)) |
| Negative   | 100 (57.8 (50.0 to 65.2)) | 271 (72.2 (67.4 to 76.7)) | 419 (72.4 (68.5 to 75.9)) | 368 (76.2 (72.1 to 79.9)) | 167 (70.8 (64.5 to 76.4)) | 1,325 (71.8 (69.7 to 73.8)) |

Abbreviations: HPV, human papillomavirus; HrHPV, high-risk human papillomavirus; LiHPV, low-risk human papillomavirus.
The overall prevalence of HPV infection was 28.2% (95% CI, 26.1% to 30.3%). We found an HR HPV prevalence rate of 24.8% (95% CI, 22.8% to 26.8%). This was similar to the 25.4% prevalence rate found by Traore et al,17 who identified high-risk types through PCR in a sample of 181 women age 20 to 56 years in Burkina-Faso.

**TABLE 4.** Prevalence of Specific HPV Genotypes by Age Group Among Women Age 25 Years or Older Attending a Community-Based Cervical Cancer Screening Program at Mont-Amba Health Centre, Kinshasa, Democratic Republic of the Congo (2015 to 2017; N = 1,846)

| HPV Type | 25-29 Years (n = 173) | 30-39 Years (n = 375) | 40-49 Years (n = 579) | 50-59 Years (n = 483) | ≥ 60 Years (n = 236) | Total (N = 1,846) |
|----------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------|
|          | No. | % (95% CI) | No. | % (95% CI) | No. | % (95% CI) | No. | % (95% CI) | No. | % (95% CI) | No. | % (95% CI) |
| HPV6     | 2   | 1.2 (0.1 to 4.1) | 4   | 1.1 (0.2 to 2.7) | 5   | 0.8 (0.2 to 2.0) | 2   | 0.4 (0.0 to 1.4) | 1   | 0.4 (0.0 to 2.3) | 14  | 0.7 (0.4 to 1.2) |
| HPV11    | 1   | 0.6 (0.0 to 3.1) | 5   | 1.3 (0.4 to 3.0) | 1   | 0.1 (0.0 to 0.9) | 1   | 0.2 (0.0 to 1.1) | 1   | 0.4 (0.0 to 2.3) | 8   | 0.4 (0.1 to 0.8) |
| HPV16    | 3   | 1.7 (0.3 to 4.9) | 7   | 1.8 (0.7 to 3.8) | 18  | 3.1 (1.8 to 4.8) | 11  | 0.6 (1.1 to 4.0) | 7   | 2.9 (1.2 to 6.0) | 46  | 2.5 (1.8 to 3.3) |
| HPV18    | 7   | 4.0 (1.6 to 8.1) | 13  | 3.4 (1.8 to 5.8) | 15  | 2.5 (1.4 to 4.2) | 8   | 1.6 (0.7 to 3.2) | 3   | 1.2 (0.2 to 3.6) | 46  | 2.5 (1.8 to 3.3) |
| HPV31    | 6   | 3.4 (1.2 to 7.3) | 11  | 2.9 (1.4 to 5.1) | 12  | 2.1 (1.0 to 3.5) | 10  | 2.1 (0.9 to 3.7) | 6   | 2.5 (0.9 to 5.4) | 45  | 2.4 (1.7 to 3.2) |
| HPV33    | 0   | 0.0 (0.0 to 0.0) | 1   | 0.2 (0.0 to 1.4) | 2   | 0.3 (0.0 to 1.2) | 3   | 0.6 (0.1 to 1.8) | 1   | 0.4 (0.0 to 2.3) | 7   | 0.3 (0.1 to 0.7) |
| HPV35    | 12  | 6.9 (3.6 to 11.8) | 8   | 2.1 (0.9 to 4.1) | 13  | 2.2 (1.2 to 3.8) | 13  | 2.6 (1.4 to 4.5) | 8   | 3.3 (1.4 to 6.5) | 54  | 2.9 (2.2 to 3.7) |
| HPV39    | 6   | 3.4 (1.2 to 7.3) | 7   | 1.8 (0.7 to 3.8) | 12  | 1.2 (0.4 to 2.4) | 4   | 0.8 (0.2 to 2.1) | 1   | 3.3 (1.4 to 6.5) | 32  | 1.7 (1.1 to 2.4) |
| HPV45    | 6   | 3.4 (1.2 to 7.3) | 9   | 2.4 (1.1 to 4.5) | 16  | 2.7 (1.5 to 4.4) | 11  | 0.6 (1.1 to 4.0) | 5   | 2.1 (0.6 to 4.8) | 47  | 2.5 (1.8 to 3.3) |
| HPV51    | 12  | 6.9 (3.6 to 11.8) | 9   | 2.4 (1.1 to 4.5) | 12  | 2.1 (1.0 to 3.5) | 7   | 1.4 (0.5 to 2.9) | 2   | 0.8 (0.1 to 3.0) | 42  | 2.2 (1.6 to 3.0) |
| HPV52    | 12  | 6.9 (3.6 to 11.8) | 9   | 2.4 (1.1 to 4.5) | 12  | 2.1 (1.0 to 3.5) | 7   | 1.4 (0.5 to 2.9) | 2   | 0.8 (0.1 to 3.0) | 42  | 2.2 (1.6 to 3.0) |
| HPV53    | 12  | 6.9 (3.6 to 11.7) | 12  | 3.2 (1.6 to 5.5) | 13  | 2.2 (1.2 to 3.8) | 15  | 3.1 (1.7 to 5.0) | 8   | 3.3 (1.4 to 6.5) | 60  | 3.2 (2.4 to 4.1) |
| HPV56    | 5   | 2.8 (0.9 to 6.6) | 8   | 2.1 (0.9 to 4.1) | 3   | 0.5 (0.1 to 1.5) | 1   | 0.2 (0.0 to 1.1) | 5   | 2.1 (0.6 to 4.8) | 22  | 1.2 (0.7 to 1.7) |
| HPV58    | 13  | 7.5 (4.0 to 12.5) | 17  | 4.5 (2.6 to 7.1) | 19  | 3.2 (1.9 to 5.0) | 13  | 2.6 (1.4 to 4.5) | 10  | 4.2 (2.0 to 7.6) | 72  | 3.9 (3.0 to 4.8) |
| HPV59    | 7   | 4.0 (1.6 to 8.1) | 6   | 1.6 (0.5 to 3.4) | 4   | 0.6 (0.1 to 1.7) | 7   | 1.4 (0.5 to 2.9) | 2   | 0.8 (0.1 to 3.0) | 26  | 1.4 (0.9 to 2.0) |
| HPV66    | 5   | 2.8 (0.9 to 6.6) | 6   | 1.6 (0.5 to 3.4) | 17  | 2.9 (1.7 to 4.6) | 8   | 1.6 (0.7 to 3.2) | 10  | 4.2 (2.0 to 7.6) | 46  | 2.5 (1.8 to 3.3) |
| HPV67    | 8   | 4.6 (2.0 to 8.9) | 7   | 1.8 (0.7 to 3.8) | 15  | 2.5 (1.4 to 4.2) | 8   | 1.6 (0.7 to 3.2) | 6   | 2.5 (0.9 to 5.4) | 44  | 2.3 (1.7 to 3.1) |
| HPV68    | 11  | 6.3 (3.2 to 11.0) | 17  | 4.5 (2.6 to 6.1) | 36  | 6.2 (4.3 to 8.5) | 26  | 5.3 (3.5 to 7.7) | 12  | 5.0 (2.6 to 8.7) | 102 | 5.5 (4.5 to 6.6) |

Abbreviation: HPV, human papillomavirus.

HPV prevalence studies using PCR as the validated method for HPV DNA detection in Central Africa. The strengths of this study are the large number of participants (1,846 women) who were tested for HPV as well as the method (PCR) used to identify 18 HPV genotypes on each positive sample.

**FIG 1.** Ten most prevalent human papillomavirus (HPV) types among women age 25 years or older attending a community-based cervical cancer screening program at Mont-Amba Health Center, Kinshasa, Democratic Republic of the Congo (2015 to 2017).
Globally, reported HPV prevalence in Africa ranged from 7% and 60%, without consideration of cytologic findings. 

Bruni et al reported an HPV prevalence of 24% in women with normal cytology in sub-Saharan Africa. The prevalence can be discrepant between studies because of differences in several factors, including world regions, study populations, sexual habits, and methods used for HPV testing. In this point of view, compared with hybrid capture 2, PCR assays are more sensitive and test for more HPV types than do the hybrid capture 2 assays. Previously, in Kinshasa, Ali-Risasi et al found an HPV positivity of 98.2% in a population of 55 patients presenting dysplastic lesions of the uterine cervix, including 47 (85.5%) who were HIV positive. This high prevalence was biased because of the limited number of women who were mainly HIV positive. It is well known that immunodeficiency favors the persistence and progression of HPV infection. Another study of women age 30 years and older in Kinshasa reported an overall HPV prevalence rate of 12.5%. This low prevalence rate might relate to the difference in age of inclusion.

In North Africa, a study revealed an overall HPV prevalence of 13.2% among 391 women age 18 to 65 years. The prevalence would be expected to be higher in this study. One explanation for this finding is the effect of cultural and sexual habits that may constitute protective factors by reducing the risk of acquisition of HPV among women in that community.

HPV infection was more prevalent in younger women age 25 to 29 years (42.2%; 95% CI, 34.7% to 49.9%). This finding is consistent with previous studies indicating that HPV was more prevalent at younger ages, between 20 and 29 years. We noted a second peak of prevalence (29.2%) in the group of women age 60 years and older. Kim et al also found the highest peak of 13.4% at 25 to 29 years of age and a second peak of 10.9% at 40 to 49 years. This bimodal curve of the age distribution of cervical HPV infection was previously shown by Bruni et al. According to the natural history, HPV prevalence is expected to be high in younger women and decline with age. The second peak observed in these studies is atypical. It could be explained by the lack of organized cervical cancer screening. Removal of cervical precancerous lesions detected in a screening activity could protect against acquisition of new HPV infections. Another factor contributing to high prevalence among older women could be the reactivation of latent infections due to immune senescence.

Our study revealed that most infections were high-risk types. This finding was corroborated by other authors. In our study, HPV68 was the most prevalent in all age groups. In the previously reported study by Ali-Risasi et al, HPV68 was also the most predominant genotype in their cohort of women in Kinshasa. HPV16 ranked seventh of all HPV types and was as prevalent as HPV18 and HPV66. We found a combined prevalence of 4.8% (95% CI, 3.8% to 5.8%) for HPV16 and/or HPV18. This prevalence was lower compared with other regions. In general, sub-Saharan Africa had the lowest HPV16 and HPV18 estimates. Troore et al did not find HPV16 in their series. HPV52 and HPV45 were also especially frequent in our study, compared with other data.

A high proportion of women harbored multiple HPV infections. The prevalence rate for multiple HPV infection was 8.3% (95% CI, 7.0% to 9.6%) compared with 19.8% (95% CI, 18.0% to 21.6%) for single HPV infection. At the global level, a study revealed that multiple HPV infections accounted for 3.2% of women tested for HPV. Infections with multiple HPV types were frequently found in sexually active and HIV-infected women. The fact that HPV genotypes found here may be different from those prevailing in invasive cervical cancers and in other locations in our region constitutes a limitation for this study. The overall evidence that HPV16 and HPV18 are the most prevalent worldwide cannot be overturned by our findings. The existing anti-HPV vaccines were designed on
the basis of etiologic fraction of different HPV types in cervical cancer. The fact that a type may be highly prevalent in a particular region does not in itself suggest that it is thus a serious contender to be included in vaccine formulations. The latter has to be based on the potential for a given type to cause cervical cancer. Next-generation vaccines should only be based on etiologic fraction of different HPV types prevalent in invasive cervical cancers. In that perspective, efforts should be made to identify the profile of HrHPV genotypes involved in invasive cervical cancers in our community.

The overall prevalence of HPV was high, particularly among women age 25 to 29 years. HPV68, which is not covered by any available vaccine, was the most prevalent HrHPV type encountered. This tendency is concordant with other studies carried out in the same region. The distribution of HPV genotypes among women in our population seems to be different from that of other world regions.

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AUTHOR CONTRIBUTIONS
Conception and design: All authors
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