Prevalence of Human Papillomavirus infection among Congolese Women with Normal Cervical Cytology

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Abstract: To determine the HPV infection prevalence among Congolese women with normal cervical cytology in southwestern Congo, in order to establish a data baseline necessary to improve the HPV infections prevention and screening. Between November 2010 and February 2011, cervical samples of 219 women attending the General Hospital of Loandjili who had a normal cervical smear dated less than one year in cytopathological Unit register were investigated. Liquid based cytology samples were obtained using a cytobrush. A portion of the sample was used to confirm the cytological diagnosis and another one for the HPV detection by nested-PCR and direct sequencing. Overall, 204 women were included. HPV prevalence was 23.5% (48). HR-HPV and LR-HPV infection was detected in 29 (60.4%) and 19 (39.6%) of study participants respectively. Thirteen HPV genotypes were identified. The most common genotypes were HPV16, 13 (27%), HPV70, 8 (16.6%), HPV33 and HPV6, 5 (10.4%). A decreasing trend of HPV infection was observed as the age increases, 38.6% below 30 years to 11.1% over 60 years (p=0.379). Conclusion: HPV prevalence among Congolese study women with normal cervical cytology was high. Knowledge of the type-specific HPV distribution in women with normal cytology could help to establish the frequency in the cytological monitoring of women between two screenings.

Keywords: Human papillomavirus prevalence, Normal cervical cytology, Pointe-noire, Southwestern Congo

1. Introduction

Worldwide, cervical cancer (CC) is the fourth most important female cancer despite an increasing incidence estimated at 528,000 new cases per 100,000 women [1]. More than, 85 % of this estimated new cases occur in developing countries, where it is the leading cause of cancer deaths in women and accounts for 13% of all female cancers [2]. Africa is one of the most affected regions in the world, with an estimated age-standardized incidence rate (ASR) of 42.7 in Eastern Africa and 30.6 in Central Africa [1].

In the Republic of Congo, the situation remains broadly similar to the rest of SSA. Indeed, age-standardized incidence rate is estimated at 27.2 per 100,000 women each year, making it the second most common cancer among Congolese women. However, it remains the leading cause of female cancer deaths with a mortality rate age-standardized at 17.6 per 100,000 women [3, 4].

Human papillomavirus (HPV) infection has been recognized as the main causal agent of CC and persistent infection with oncogenic genotypes is required to cause cancer [5, 6]. More than 150 HPV are well-characterized, of which 40 are known to infect the genital tract [7, 8]. Different epidemiological studies have suggested that about 50–75% of sexually active women are infected with HPV at some point of their lives [9]. However, distribution and prevalence of HPV infection rates vary largely between geographical regions worldwide [10]. Furthermore, the burden of disease is mostly shared by 2 types (HPV 16 and 18) which are commonly reported in scientific literature as responsible of 70% of lesions [11].

Knowledge related to the involvement of HPV in the development of CC has led to improved public health programs for the prevention and fight against this disease. The introduction of sensitive and specific diagnostic tests and the programs adapted screening are essential in the identification of HPV infections at a very early stage among women without cervical abnormalities. HPV data obtained on the cytologically normal women revealed important aspects of HPV infection to identify some risk population and adjust the monitoring interval relative to the Pap test. However, these data show some differences by region and age groups [12]. The HPV prevalence in women with normal cervical cytology ranges from 1.4% in Spain to 25.6% in Nigeria, and young women are the most affected [13, 14].

To our knowledge, the Congolese population is very poorly studied and no study has yet estimated the prevalence of HPV in women with normal cytology. In this Hospital-based study, we aimed to determine HPV prevalence and type distribution profile among Congolese women with normal cervical cytology in the southwestern part of the country in order to provide valuable information for health policy.
makers (in particular interval of the screening tests) about the epidemiology of this sexually transmitted infection.

2. Materials and Methods

2.1. Study population and Specimen Collection

The study was conducted at the General Hospital of Loandjili (GHL) in Pointe-Noire city. A cross-sectional study was carried out in the Medical and Morphological Analysis Laboratory (MMAL) of the GHL, between November 2010 and February 2011, from 219 women aged 21-68 years. The study focused on women attending the cytopathology unit of MMAL, referred for routine cervical smears and diagnosis with cytomorphologically normal smears dating to a year or less. Eligible women should be sexually active, not pregnant, not vaccinated against HPV, did not have previous history of cervical abnormalities until the date of sampling and their initial Pap smear was read as normal. By using the laboratory register, all women eligible for the survey period were recalled. A liquid base cytology sample was obtained for all women after informed consent and using the ThinPrep® Pap test Kit as per the manufacturer’s instruction and stored at -20°C in the PreservCyt® Transport medium tubes (ThinPrep Pap Test, Cytex Corporation, Marlborough, Ma. USA). The study had the agreement of the Congolese ethics committee for Research in Health Sciences.

2.2. Cytological Diagnosis

To confirm the diagnosis, a slide smear was performed for all samples and interpreted by the Head of the Cytopathology Unit of the GHL. All abnormal cytology results were excluded from the molecular study.

2.3. Molecular study

Molecular study was performed with five mL of residual ThinPrep® liquid based cytology samples in the laboratory of Virology, Microbiology and Quality/Eco-toxicology and Biodiversity (LVMQ/ETB) at Faculty of Sciences and Technologies, Hassan II University in Morocco.

HPV Detection and Typing

DNA extraction was performed with the Phenol/chloroform extraction protocol after enzymatic digestion with proteinase K as previously described [15]. HPV-DNA detection were performed by nested-PCR using MY09/MY11 and GP5+/GP6+ consensus primers [16] and genotyping were performed by direct sequencing [17] using the BigDye terminator technology according to the manufacturer’s protocol. Sequence analysis was performed using the on-line BLAST program according to the technique described by Lee in 2009. The hypervariable, 34-50 bp DNA (hypervariable sequence) sequence downstream of the GP5+ primer site was compared to the known HPV DNA sequences in the GenBank database (NCBI, National Institutes of Health, Bethesda, MD, USA) [17]. The use of this sequence (34 to 50 bp) of L1 gene could help to identify any HPV genotypes [18]. The DNA concentration was measured by a NanoDrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, USA). The DNA quality and the absence of PCR inhibitors were assessed by amplifying a 268 bp fragment of the housekeeping β-globin gene using the GH20 (5’GAA GAG CCA AGG ACA GGT AC3’; nt 54–73) and PC04 (5’CAA CTT CAT CCA CGT TCA CCG3’; nt 195–176) primers [19]. All amplification was carried out with 100ng/μl of DNA concentration in a Perkin Elmer 2400 GeneAmp PCR thermal Cycler (Scientific Support, Inc, Hayward, CA). DNA from the SiHa cell line (one to two viral copies of HPV16 DNA/cell) was used as positive PCR control and Ultra-pure PCR water (Bioline, UK) as negative control to assess the success of the amplification.

Data Analysis

Results were analyzed statistically by the chi-square test or Fisher's Exact test (when certain values to be compared were lower than 5) using the OpenEpi version 3.01 software (http://www.openepi.com). Statistical significance was defined as p < 0.05 for all tests.

3. Results

The mean age of enrolled participants was 40.8±10.9 years (range: 21-68)

3.1. HPV Detection and Genotyping

Two hundred and four (93.2%) out of 219 enrolled women were normal cervical cytology confirmed and 15 (6.8%) were excluded because having cytological abnormalities in proofreading their cervical exams. HPV-DNA was positive in 48 (23.5%) out of 204 samples, of which 29 (60.4%) were infected with high-risk HPV (HR-HPV) genotypes and 19 (39.6%) with low-risk HPV (LR-HPV) genotypes (p=0.066). These proportions represented 14.2% (HR-HPV) and 9.3% (LR-HPV) by considering the entire study population.

Thirteen different HPV genotypes were identified as shown in Figure 1. The most common type was HPV16, 13 (27.0%) followed in descending order by HPV70, 8 (16.6%), HPV33 and HPV65 (10.4%), HPV31, HPV35 and HPV81, 3 (6.2%), HPV58 and HPV83, 2 (4.2%) and HPV18, HPV67, HPV66 and HPV54, 1 (2.0%) respectively.

3.2. HPV infection according to the Age group

The age-specific prevalence of HPV detection is reported in Table 1. HPV-DNA was detected in 31.8% (7/22) of women aged less than 30 years (42.8% were HR-HPV), with prevalence decreasing to 11.1% (1/9) among women older than 60 years (31.8% vs. 11.1%; p=0.379). No significant difference was also observed between the age group <30 years and the other 3 groups (p = 0.74). Low-risk HPV were identified in women less than 50 years, while high-risk HPV were identified in all of the age groups, with an exclusive among women aged over 50 years (Figure 2).
4. Discussions

To date, few studies have evaluated the prevalence of HPV infection in women with normal cervical cytology in many sub-Saharan African countries, one of the areas with a high incidence of cervical cancer [20]. We report here the first investigation of its kind done in Southwestern Congo in the aim to provide a comprehensive description of HPV genotypes among women with normal cervical cytology. The present study has highlighted the HPV type’s distribution profile among Congolese women in the southwestern part of the country particularly in the Pointe-Noire city. Out of 219 cervical samples collected according to inclusion criteria, 204 were confirmed to have a normal cervical cytology and were included, accounting for just over 93% of the starting sample. Viral DNA was detected in 23.5% of cases, which is similar to that reported in other high-risk populations in SSA specially in Nigeria (23.7%), Zimbabwe (24.7%), Benin (26.7%), Mozambique (32.1%) and Uganda (35.8%) [13, 21, 22] in women without abnormal lesions.

However, this prevalence found in our study was high compared to the world average which is 10.4% [23] and that of most developed countries in the Americas (13.0%), Europe (8.1%) and Asia (8.0%) [13] but, lower than the percentage found in Honduras (51%) [24]. As in most developing countries, some socio-cultural factors may be related to the high prevalence of HPV infection among women with normal cytology in this study, particularly the lack of education, early age of sexual intercourse, multiple sexual partners, multiparity, the high prevalence of sexually transmitted infections and poverty [20, 25, 26]. Indeed, cultural habits and sociodemographic characteristics of the population in southwestern Congo are in favor of a high-risk HPV infection. Moukassa et al. (2007) showed in his study that, in this part of the country, the age of first sexual intercourse was 16 years [27].

Our study showed that infections caused by high-risk HPV types were predominant (60.4%) compared to those caused by low risk HPV types (39.6%). HPV16 (27.0%) and HPV70 (16.6%) were the most prevalent among the high- and low-risk types, respectively. On the other hand, HPV18, 67, 66 and 54 were identified as less prevalent (2%) in the study participants. The second most common type of high- and low-risk HPV detected were HPV33 and HPV6 (10.4%) respectively. Overall, the type-specific HPV profile reported in our study may appear different from any other region of the world [13, 28, 29], thus confirming the data on the prevalence of HPV reported by IARC showing that viral prevalence can differ almost 20 times between regions [12, 20]. However, HPV16 remains the most prevalent type found in our study, which is in agreement with several studies worldwide [13-15, 23, 28, 30-33].

Taking into account the oncogenic HPV covered by current vaccines, HPV18 is the fifth type with only 2% of infections in our study. Secondly, we noted the absence of HPV11 in our study population, one of main low-risk HPV responsible of over 90% of the benign lesions with HPV6 [34].

The age pattern of HPV prevalence in this study showed that nearly 32% of women aged less than 30 years were HPV positive, of which 42.8% were high-risk HPV infection. We have observed also a decrease trend in the prevalence according to age, to 11% among women aged over than 60 years. No significant difference was observed, despite the decreasing trend of infection with age, probably due to the small size of our sample (p=0.379). However, these results confirm that, young women are more vulnerable to HPV infection than the oldest as reported in other studies of cytologically normal women [13, 24]. The youngest age group (<30 years) with a higher incidence of HPV infection may be an indicator of the infection transmission following an active sexual life [25]. However in ours study, we did not find a second peak of infection in older women, as reported in other studies [13, 20, 24, 35].

The high prevalence of infection found in our study attests to the seriousness of the problem at the national level. In most SSA countries, access to health services is limited and programs organized screening of cervical cancer are lacking. A good effective health system with the necessary equipment and trained providers is essential for prevention, screening, diagnosis and treatment with appropriate monitoring program for the most vulnerable populations.

However, beyond the fact that this study provides important data derived from a poorly studied population, it nonetheless has some limitations that do not allow us to extrapolate the results to the national level. The small sample size limits our results to our study area. We have not been able to establish the co-infections that may partly explain the risk to our population studied in develop higher grade lesions.

However, the distribution profile of specific HPV types in this study may have a positive impact the effectiveness of current vaccines in the Congolese population that should be elucidated in future studies.

5. Conclusion

HPV infection was high in our study population, particularly high-risk HPV infection. Given the lack of public awareness about HPV infections, these findings may provide a better estimate of the screening interval in normal cervical cytology women in this part of the country. This study therefore, provides preliminary guidance in the implementation of prevention strategies against HPV infections and its complications.

6. Acknowledgements

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7. Competing interests

The authors declare that they have no competing interests.

8. Authors’ contributions

ALMB conducted all handling and the overall design of the experiment. MM has participated greatly in handling and MME were responsible for the implementation of the project and MME was also the supervisor. All authors read and approved the final manuscript.

References


Figure 1: Prevalence of specific-types HPV in women with normal cytology evaluated in Pointe-Noire, Southwestern Congo.
Figure 2: General trend of HPV and that of HR-HPV infection based on age among Southwestern Congolese women with normal cervical cytology

Table 1: Distribution of HPV infection by age among 204 women with normal cytology

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of patients</th>
<th>All HPV +</th>
<th>HR-HPV</th>
<th>LR-HPV</th>
</tr>
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<tr>
<td></td>
<td>n (%)</td>
<td>HPV16</td>
<td>HPV33</td>
<td>HPV31</td>
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<tr>
<td>&lt;30</td>
<td>22</td>
<td>7 (31.8)</td>
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<td></td>
</tr>
<tr>
<td>30-39</td>
<td>81</td>
<td>22 (27.1)</td>
<td>6 (27.3)</td>
<td>1 (4.5)</td>
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<td>40-49</td>
<td>59</td>
<td>12 (20.3)</td>
<td>2 (16.6)</td>
<td>2 (16.6)</td>
</tr>
<tr>
<td>50-59</td>
<td>33</td>
<td>6 (18.2)</td>
<td>2 (33.3)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>≥60</td>
<td>9</td>
<td>1 (11.1)</td>
<td>1 (100)</td>
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<td>Total</td>
<td>204</td>
<td>48 (23.5)</td>
<td>13 (27.0)</td>
<td>5 (10.4)</td>
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</tbody>
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(*), Unidentified

Author Profile

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