Oncogenic human papillomavirus infection and genotypes characterization among sexually active women in Tenkodogo at Burkina Faso, West Africa

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ABSTRACT

Objective: This study was conducted to determine the prevalence and distribution of high-risk human papillomavirus (HR-HPV) genotypes among sexually active women in Tenkodogo, Burkina Faso.  
Methods: Among 131 sexually active women attending the Tenkodogo Urban Medical Center, endocervical samples were collected prior to screening for precancerous lesions. After viral DNA extraction, fourteen HR-HPV genotypes were characterized by real-time multiplex PCR in these cervical samples.  
Results: The mean age was 35.5 ± 9.5 years. Of the 131 women, 45 were infected with at least one HR-HPV genotype. The prevalence of HR-HPV infection among these women was 34.4%. Among the 45 oncogenic HPV-infected women, single HR-HPV genotype was found in 55.6% while 44.4% were infected with more than one HR-HPV genotype. The most frequent genotypes were HPV56 (36.5%), HPV66 (36.5%).  
Conclusion: Tenkodogo women included in this study had a higher prevalence of HPV 56, HPV 66. A larger study with a more representative sample would therefore be needed to determine predominant oncogenic genotypes in the subregion and especially in cancer cases.

1. Introduction

Oncogenic HPV (HR-HPV) genotypes are closely related to malignant tumors. Sexually active women are at risk of genital HPV infection and the persistence of this infection is necessary for the development of cervical cancer \textsuperscript{[1,2]}, which is the leading cause of cancer death among women in Sub-Saharan Africa. HPV-related cancers in women remain a real public health problem. Currently available bivalent and quadrivalent vaccines target only HPV 16 and 18, leading causes of cervical cancer worldwide. The nonavalent vaccine detects additional high-risk genotypes (HPV16, 18, 31, 33, 45, 52, and 58). HPV studies in Burkina Faso were limited to two regions (central and the high basins) and had shown diverse predominance of HR-HPV genotypes in women by region \textsuperscript{[3–8]}. Other genotypes would be more common in the African population, particularly in Burkina Faso \textsuperscript{[4,7,8]}, hence it is of importance to characterize HR-HPV circulating in order to consider adequate prevention strategies. The objective of this study was therefore to contribute to the identification of HR-HPV circulating in the health region of eastern central Burkina Faso, particularly in the city of Tenkodogo.

2. Methods

2.1. Site and study population

This cross-sectional study was conducted in Tenkodogo (located in the health region of eastern central Burkina Faso). From April to May 2016, at the Urban Medical Center of Tenkodogo (CMU), 131 sexually active and consenting women provided cervical samples for this study. Only non-pregnant sexually active women in non-menstrual period who had not undergone a total hysterectomy were included in the study.

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Each woman answered to a questionnaire that collected information on socio-demographic, behavioral and sexual characteristics, level of knowledge on HPV and cervical cancer.

2.2. Endocervical samples collection

Samples have been taken through endocervical swabbing of the uterus using a sterile cotton swab and a single use speculum. The samples thus obtained have been immersed in a transport medium which was provided with DNA-Sorb-A kit (Sacace Biotechnologies, Como, Italy) and kept at at −20 °C in the medical analysis laboratory of Regional Hospital (CHR) of Tenkodogo, then at the Pietro Annigoni Biomolecular Research Center (CERBA) of Ouagadougou until the DNA extraction.

2.3. Screening for precancerous lesions

Immediately after sampling, screening for precancerous lesions was done for the women by visual inspection with acetic acid and Lugol’s iodine (VIA/VIL).

2.4. Ethics approval and consent to participate

This study was approved by the Ethics Committee for Health Research of Burkina Faso (Deliberation number 2016–3-026) as well as that of the Regional Office of Health (DRS) of the Central-East Health Region. All participants gave their free and written consent to participate in the study. Confidentiality and anonymity with respect to the information collected were required.

2.5. HPV DNA extraction

The viral DNA was extracted using the DNA-Sorb-A kit (Sacace Biotechnologies, Como, Italy) following the protocol supplied by the manufacturer.

2.6. Determination of HPV genotypes by real time PCR

Characterization of HR-HPV genotypes, was performed using Sacycler-96 Real Time PCR v.7.3 (SACACE Biotechnologies®, Como, Italy) and the “HPV Genotypes 14 Real-TM Quant” kit (Sacace Biotechnologies, Como, Italy), which allowed us to detect fourteen genotypes of HR-HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) using multiplex real time-PCR for each sample with the β-globin gene as internal control. The PCR program used was as follows: 1 cycle of 95 °C for 15 min; 5 cycles of 95 °C for 05 s, 60 °C for 20 s, 72 °C for 15 s; 40 cycles of 95 °C for 05 s, 60 °C for 30 s, and 72 °C for 15 s.

2.7. Data analyses

Data were recorded and analyzed using IBM SPSS V.21 (SPSS, Chicago, Illinois, USA) and Epi Info 6. The Chi-square test was used for comparisons with a significant difference for p < 0.05.

3. Results

During the enrollment period in the Department of Obstetrics and gynecology, 135 sexually active women gave their consent to participate in this study. The main motivation for consultation was to take advantage of the cervical cancer screening, symptoms of leucorrhrea and pruritus, abdominal pelvic pain, dysmenorrhea, menstrual cycle disorders, requirement of contraception and fertility.

3.1. Sociodemographic characteristics of the study population

The mean age of women in our study was 35.5 ± 9.5 years (20–60) and the median was 35 years old. Women older than 35 years were significantly more represented (53.4% or 70/131). Married women or lived with a partner were 87.0% (114/131); 55.0% were illiterate (72/131), 55.7% (73/131) were working in the informal sector and only 12.2% (16/131) were students or civil servants. The age at first intercourse ranged from 14 to 30 years with an average of 18.8 ± 2.1. Only one woman reported having more than one sexual partner; the others had only one. The majority of women (96.2%) had been pregnant at least once in their lifetime and 43.5% had at least 5 children. More than half of women (63.4%) used a contraceptive method. In addition, 79.4% had never used a condom during intercourse and 80.9% had no knowledge of cervical cancer and Human Papillomavirus infection at the time of inclusion in this study (Table 1).

3.2. Prevalence of HR-HPV infection and genotypes characterization in women in Tenkodogo

The amplification kit used allowed us to identify fourteen HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) among which we found 12 genotypes in women in Tenkodogo. HPV 39 and HPV 59 were not found. Among 131 women, 45 were infected with at least one HR-HPV genotype. The prevalence of HR-HPV infection among these women was 34.4% (CI = 26.4–43.2). The total number of genotypes per infected woman ranged from 1 to 5. Considering multiple infections, we counted a total of 74 HR-HPV genotypes. Of these genotypes, the most frequent were in descending order HPV56 (36.4%), HPV66 (36.4%) followed by HPV68 (5.4%) and HPV51 (5.4%). The frequency of other genotypes ranged from 1.3% to

| Characteristics                      | Number (n = 131) | Percentage (%) |
|--------------------------------------|-----------------|---------------|
| **Age group in years**               |                 |               |
| 20–24                                | 14              | 10.7          |
| 25–29                                | 29              | 22.1          |
| 30–34                                | 18              | 13.8          |
| ≥ 35                                 | 70              | 53.4          |
| **Level of education**               |                 |               |
| Illiterates                          | 72              | 55.0          |
| Primary                              | 28              | 21.3          |
| High school                          | 31              | 23.7          |
| **Marital status**                   |                 |               |
| Married or lives with a partner      | 114             | 87.0          |
| Single                               | 5               | 3.8           |
| Widow                                | 9               | 6.9           |
| Divorced                             | 3               | 2.3           |
| **Number of sexual partner**         |                 |               |
| 1                                    | 130             | 99.2          |
| ≥ 2                                  | 1               | 0.8           |
| **Occupation**                       |                 |               |
| Housewives                           | 42              | 32.1          |
| Pupils                               | 7               | 5.3           |
| Civil servants                       | 9               | 6.9           |
| Informal sector                      | 73              | 55.7          |
| **Use of contraception**             |                 |               |
| No/Natural method                    | 48              | 36.6          |
| Yes                                  | 83              | 63.4          |
| **Use of condom**                    |                 |               |
| Yes                                  | 27              | 20.6          |
| No                                   | 104             | 79.4          |
| **Age at the first sexual intercourse** |           |               |
| 14–17                                | 38              | 29.0          |
| 18–24                                | 92              | 70.2          |
| ≥ 25                                 | 1               | 0.8           |
| **Number of pregnancies**            |                 |               |
| 0                                    | 5               | 3.8           |
| 1–5                                  | 85              | 64.9          |
| 5–11                                 | 41              | 31.3          |
| **Knowledge on HPV and cervical cancer** |         |               |
| Yes                                  | 25              | 19.1          |
| No                                   | 106             | 80.9          |
4.1% as shown in Table 2. Among the 45 women infected by the oncogenic HPV, 55.6% (25/45) were infected by only one genotype from HR-HPV and 44.4% (20/45) infected by more than one HR-HPV genotype. The number of HR-HPV genotypes in the multiple infection cases was 49 (66.2%) versus 25 (33.8%) involved in single infections (Table 2). Considering the genotypes covered by the HPV vaccines, 3% (4/131) women had HPV 16 and/or 18, or 9% (4/45) of those infected with at least one HR-HPV genotype; 7.6% (10/131) had one or more of 16/31/33/45/52/58 genotypes or 22.2% (10/45) of those infected.

### 3.3. Potential risk factors associated with the carrying of HPV

In Table 3, we analyzed association between the HR-HPV infection and age, age of first sexual intercourse, level of education, marital status, number of sexual partner, frequency of sexual intercourse, occupation, use of condom, parity, use of contraception, knowledge on HPV and cervical cancer, and VIA/VILI result. Women older than 55 years and those between age 40 – 44 years were less infected (2.2%), the highest carrier rate of HR-HPV (24.4%) was found among the 25–29 age group (Table 3). Visual screening for precancerous lesions showed that 2 of the 131 women or 1.5% were positive for VIA/VILL. Of these two women, one was carrier of HPV as shown in Table 3. This woman was infected with HPV66. Among the 45 women infected with at least one HR-HPV genotype, 88.9% (40/45) had more than two sexual intercourse per month against 6.7% (3/45) with a sexual intercourse per month, while 4.2% (2/45) had two sexual intercourse per month. This study found no statistical association between HR-HPV infection and the risk factors analyzed.

### 4. Discussion

Voluntary inclusions have been a limitation of the study and therefore subject to selection bias. This may justify the small size of the study cohort and the high prevalence of the HPV 56 and 66 genotypes.
Benin [11]. On the other hand, it was lower than those of 75.0% in Ghana [12], 74.0% in Tanzania [13] and 54.0% in South Africa [14], 41.5% in Burkina Faso [7] obtained from studies of younger populations with a mean age of less than 30 years while that of our study was 35.5 ± 9.5 years.

Although the prevalence of genital HPV infection varies by region around the world [15], all studies agree that it is particularly important in young women at the onset of sexual activity and decreases later when age increases because of both clearance and the acquisition of immunity. Our prevalence could be justified by the mean age of women which was higher than that of the study populations of the last three authors. However, our prevalence was higher than 25.4% in Burkina Faso [6], 24% [16], 15.9% in Great Britain [2] and 7.8% in Italy [17].

In this study, the most common genotypes among women in Tenkodogo were HPV 56 (36.5%) and HPV 66 (36.5%) followed by HPV 68 and HPV 51 with a prevalence of 5.4% for each. The least common genotypes in descending order were HPV 18, 35, 31, 58, 52, 45, 33, and 16. HPV 59 and 39 were not found. Similar studies on HPV conducted in Burkina Faso and Vietnam using the same amplification kit and the same device showed various and predominant genotypes other than 56 and 66 [5–7,18–20].

Among women recruited in Tenkodogo, HPV genotypes 16 and 18 covered by currently vaccines available in Burkina Faso, accounted for 4.1% and 1.4% respectively. The prevalence of HPV18 obtained is lower than that of 5.5% in Great Britain [2], 10.6% in Ghana [12], 14.8% in Bobo-Dioulasso [6], 14.3% [8] and 5.1% [7] in Ouagadougou. The results of these studies thus show a regional difference in the distribution of HPV 18. The low prevalence of single HPV 16 (1.4%) infection would be contrary to the literature according to which genotype 16 is the most common in the world, especially in Europe, [15,22], in the United States [23] and in North Africa [24]. On the other hand, our results corroborate those of some studies that had already shown a low prevalence of HPV 16 in some African countries compared to other HR-HPV genotypes [4–8,25]. Although HPV 16 appears to be the most prevalent in the world and particularly in cases of cervical cancer, studies in the African subregion have shown the existence of other predominant genotypes. In Burkina Faso, the study by Zohoncon and al. (2016b) [19] on invasive cervical cancer had revealed a predominance of HPV 18, 31, 39, 16, 45, 35 and 58. In addition, two similar studies conducted in Benin and Burkina Faso in High-Grade Cervical Intraepithelial Neoplasia and in Cervical Cancer showed lack of HPV 16 and a predominance of oncogenic genotypes, 18, 45, 35, 52, 33, 51, 31, 58, 66, 68, 56, 59, 68 [18,19]. This classical difference in the distribution of genotypes in our study population should lead to a larger study with more representative sample, to determine if the frequency of HPV16 circulating in women is lower than other populations and to characterize the predominant oncogenic genotypes in the subregion and particularly in cervical cancer.

Among the infected women in our study, we found 44.4% of women who had two or more types of HR-HPV. In contrast, 55.6% (25/45) was infected with a single HR-HPV genotype. Our prevalence of 44.4% multiple infections in the Tenkodogo HR-HPV positive women population is similar to the 48.1% reported by Kavanagh and al. [21] but is higher than that of 12.3% reported by Ouedraogo and al. [8] and 15.2% found by Traore and al. [6]. The number of isolated HR-HPV genotypes among infected women ranged from 2 to 5 in multiple infections. This result is comparable to those of Traore and al. [6] and Ouedraogo and al. [7] who found a variation of 2–3 and 2–5 h-HPV genotypes respectively in multiple infections. We did not find HPV 16 and HPV 18 coinfection and our results corroborate those of Traore and al., Ouedraogo and al., and Pannatto and al. [6,7,22].

4.2. HR-HPV carriage and risk factors associated with infection

In this study, the carrying of the HR-HPV was not associated with risk factors such as the frequency of sexual intercourse [26], the age, the age of first sexual intercourse, the level of education, the marital status, the number of sexual partner, the occupation, the use of condom, the parity, the use of contraception, the knowledge on HPV and cervical cancer, or the VIA/VILI result. This lack of statistically significant association could be explained by the small size of our study population. The prevalence of precancerous lesions detected by VIA/VILI was 1.5%. This value is lower than that of 6% and 4.2% obtained respectively for women in Ouagadougou and Bobo-Dioulasso [6,7]. HR-HPV infection was found in 50.0% of women with a positive VIA/VILI result.

Studies have shown a prevalence of HR-HPV infection of 73.5% and 41.5% in women aged 10–25 years [13] and 15–19 years old [7]. In this study, we found that HR-HPV infection was higher in women aged 25–29 years (24.4%). This high prevalence of HR-HPV infection could be explained by the fact that 99.2% of women had their first sexual intercourse between 14 and 24 years of age and 79.4% had never used a condom during sex. As pointed out by Gavillon and al., 2010, this prevalence is inversely proportional to the age of the patient [27].

Among 45 women infected with at least one HR-HPV genotype, 88.9% (40/45) of women had more than two sexual intercourse per month. We did not find a statistically significant association between carriage of HR-HPV and frequency of sexual intercourse (p < 0.26). Our results corroborate those of Ouedraogo and al. [7] who did not find this correlation in teenage girls in Ouagadougou. However, our result differs from that of Figueroa and al. [26] who found a highly significant association between high prevalence of HPV and frequent sexual activity.

The results of this study could not be generalized to the entire population of Tenkodogo but they helped to know the HPV genotypes present in a population of women in this city. Our results show that it would be important to extend HR-HPV studies in all regions of Burkina Faso in more representative populations to provide data on the distribution of genotypes within populations.

5. Conclusion

Tenkodogo women included in this study had a higher prevalence of HPV 56, HPV 66, followed by oncogenic genotypes 68, 51, 18, 35, 31, 58, 52, 45, 33 and 16. HR-HPV other than HPV16 and HPV 18 were frequently found in studies in Burkina Faso and oncogenic genotypes 56 and 66 seem to be the most predominant in our study population. A larger study with a more representative sample would therefore be needed to determine predominant oncogenic genotypes in the subregion and especially in cancer cases.

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Competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.
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