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Molecular Epidemiology of High-Risk Human Papilloma Virus Infection in Sexually Active Women at Bobo-Dioulasso University Teaching Hospital

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Abstract

The objective of this study was to determine the prevalence of HPV-HR genotypes in the population of sexually active women in Bobo-Dioulasso, Burkina Faso. **Methods:** This study took place at Souro Sanou Teaching Hospital in Bobo-Dioulasso from September to June 2017. A total of 234 women in the gynecological period and also sexually active were enrolled after they gave an individual consent. Swabbing of the endocervical canal was done. From the sample stored at −20˚C, the viral DNA was extracted using the "DNA-Sorb-A" kit from SACACE biotechnologies®. Amplification of the PCR of the extracted DNA was made, using the "HPV Genotypes 14 Real-TM Quant" V67-100 FRT kit. Data were analyzed with SPSS software version 17.0 and Epi Info 6. Chi-square and Fisher's tests were used to compare proportions and averages; a link was significant when $p < 0.05$. **Results:** The mean age was 30.7 ± 7.3 years (median: 30 years); 84.5% of them were married, 43.5% had a socio-professional activity and 61% were schooled. A total of 20.6% of women were positive for at least one of the following HPV-HR genotypes: HPV 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Genotypes the most frequently found were HPV 52 with 11 cases (17.2%) and 66 with 10 cases (15.6%). **Conclusion:** Our results show a predominance of HPV-HR genotypes not covered by available vaccines. Mapping genotypes are needed to fully understand viral ecology at the national level. But for instance, the nonavalent vaccine, which has a better coverage of the predominant genotypes, is to be promoted.

Keywords

HPV-HR, Epidemiology, Genotypes, Bobo-Dioulasso

1. Introduction

Human papillomavirus (HPV) genital infection is considered to be the most common sexually transmitted infection (STI) worldwide [1]. There are more than 100 genotypes, some of which are known as high-risk oncogenes (HPV-HR) and others as low risk. Among HPV-HR, genotypes 16 and 18, responsible for about 70% of cervical cancer cases, are the preferred targets for HPV vaccines [2]. These prophylactic vaccines initially targeted genotypes 16 and 18 (bivalent vaccine), and 6, 11, 16 and 18 (tetravalent). Genotypes 6 and 11, low oncogenic risk, are responsible for more than 90% of condylomata also called anogenital warts. HPV-HR, other than 16 and 18, is responsible for 30% of cervical cancers. In recent years, a nonavalent vaccine, incorporating some of these high-risk genotypes, has emerged in the market targeting genotypes 6, 11, 16, 18, 31, 33, 45, 52, 58. Three vaccines are available on the market. At a time when our resource-limited countries are initiating HPV vaccination policies, it is important to know the epidemiology of circulating genotypes in our populations for a better analysis of the expected results. This study aimed to determine the prevalence of HPV-HR genotypes in a population of sexually active women in Bobo-Dioulasso, Burkina Faso.

2. Methodology

2.1. Study Population

The study was conducted from September to June 2017 in the Department of Obstetrics and Gynecology and Reproductive Medicine at Bobo-Dioulasso Teaching Hospital in Burkina Faso and enrolled 234 women. Non-pregnant women and girls who presented themselves in a gynecological consultation, freely consenting after information about the interest of the study, were included. Women and girls who are virgins or who have had a hysterectomy have not been included.

2.2. Study Procedures

Each woman benefited from an endocervical swab. The sample thus collected was introduced into a transport medium and stored at −20˚C pending the extraction of the DNA. Then a visual inspection of the cervix after application of acetic acid (IVA) and lugol (IVL) was performed. The observed results were transcribed on the data collection sheet. Follow-up and/or care/treatment was offered to women based on IVA/IVL results according to the national protocol.

Extraction of viral DNA from HPV was done using the "DNA-Sorb-A" kit

from SACACE biotechnologies®. Real-time PCR amplification of DNA extracted from endocervical samples was performed to search for human papillomaviruses. This PCR was done using the "HPV Genotypes 14 Real-TM Quant" V67-100 FRT kit (SACACE biotechnologies®, Italy) which makes it possible to detect 14 high-risk human papillomavirus genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and following the protocol provided by the manufacturer.

2.3. Statistical Analyses

The data was processed and analyzed using SPSS software version 17.0 and Epi Info 6 software. Chi-square or Fisher tests were used to compare proportions and averages. All the statistical tests of our analysis were considered significant for a threshold of $p < 0.05$.

3. Results

3.1. Sociodemographic Characteristics

Sociodemographic characteristics are shown in Table 1. The mean age of women in our study was 30.7 ± 7.3 years with a median of 30 years; and 57.7% of women were in the 20 to 30 age group. These were married women in 84.5% of the cases and 43.5% of the women had a socio-professional activity. In 61% of cases, women were educated. Women with less than secondary education level accounted for 39.9% of educated women.

3.2. Prevalence of HPV-HR Infection

Women who were positive for at least one HPV-HR genotype were 48 out of 234, a prevalence of 20.6%.

The kit used made it possible to characterize 12 genotypes of HPV-HR. These were HPV 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

By accumulating HPV-HR genotypes, given that some women were infected with more than one genotype, the total number of genotypes found was 64. Table 2 shows the distribution of women by HPV-HR genotypes isolated.

In the 48 HPV-HR positive women, the number of genotypes per woman ranged from 1 to 5. The number of multiple infections was 11 (22.92%).

The frequencies of the genotypes found are reported in Figure 1.

The most common genotypes were HPV 52 with 11 cases (17.2%) and 66 with 10 cases (15.6%). HPV 18 was found in 4 cases (6.3%) and HPV 16 was never found.

3.3. Prevalence of VIA/VILI Abnormalities

VIA/VILI was abnormal in 16 women (7.0%). The results of HPV-HR carriage are reported in Table 3.

Of these 16 women with VIA/VILI abnormalities, 10 had at least one HPV-HR genotype and none of these had HPV 16 or 18.

Table 1. Sociodemographic characteristics of women (n = 234).

Characteristics	Proportion
Age (years)	
Mean (DS)	30.7 (7.3)
≤ 19, n (%)	5 (2.1)
20 - 25, n (%)	65 (27.8)
26 - 30, n (%)	70 (29.9)
31 - 35, n (%)	39 (16.7)
36 - 40, n (%)	35 (15.0)
41 - 45, n (%)	11 (4,7)
46 - 50, n (%)	8 (3,4)
≥51 n (%)	1 (0,4)
Marital status, n (%)	
Married	198 (84.5)
Single	33 (14.0)
widowed	3 (1.5)
Education level, n (%)	
Illiterate	91 (39.0)
Primary	57 (24.0)
Secondary	75 (32.0)
University	11 (5.0)
Age at first intercourse (years)	
Mean (DS)	18.2 (1.8)
<19, n (%)	147 (62.8)
19 - 30, n (%)	86 (36.8)
>30, n (%)	1 (0.4)
Condom use by the partner, n (%)	
Never	177 (76.0)
Sometimes	26 (11.0)
Rarely	19 (8.0)
Every time	12 (5.0)

3.4. HPV-HR Infection Carriage by Age and Genotype

The distribution of HPV-HR infection by age and genotype is shown in Table 4.

The number of infected women up to age 30 was 34 out of 140 (24.3%), and 14 out of 94 after 30 years (14.9%) (p = 0.8).

The number of women with multiple genotype infections was 11, a prevalence of 4.7% of the study population, and a proportion of 22.9% of infected women. The prevalence of multiple infections was 7.9% for women less than or equal to

Table 2. Distribution of women by isolated HPV-HR genotypes.

Genotypes HPV-HR	Number of women	Percentage
18	2	4.17
31	3	6.25
39	3	6.25
45	2	4.17
51	3	6.25
52	**7**	**14.58**
56	2	4.17
58	2	4.17
59	2	4.17
66	**8**	**16.67**
68	3	6.25
35 + **52**	2	4.17
39 + 68	2	4.17
68 + 51	1	2.08
56 + **66**	1	2.08
18 + 59	1	2.08
35 + **66**	1	2.08
68 + 51 + **52**	1	2.08
18 + 39 + **52**	1	2.08
31 + 35 + 51 + 56 + 58	1	2.08
Total	**48**	**100.0**

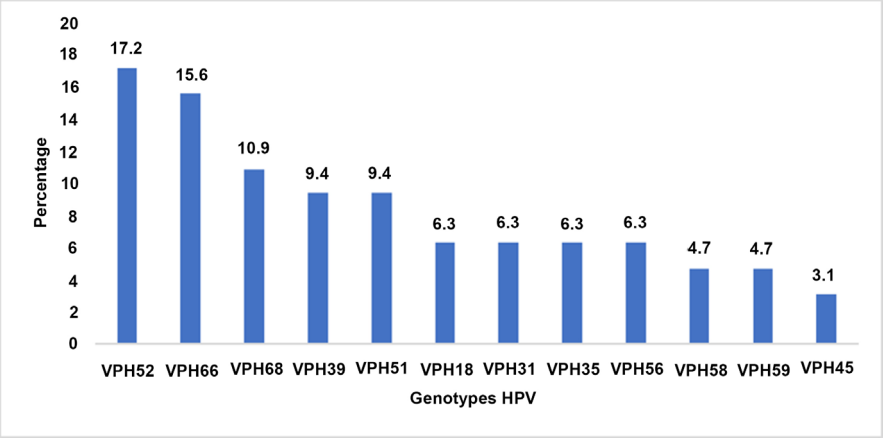

Figure 1. Frequency of isolated genotypes in infected women (n = 64).

30 years of age and 2.1% for women over 30 years of age (p = 0.128). No multiple infections were found after 40 years.

Table 3. HPV-HR carriage according to VIA/VILI results.

HPV-HR carriage	VIA/VILI		Total	p
	Positive	Negative		
Positif	10	38	48	
Negatif	6	180	186	0.8
Total	16	218	234	

Table 4. Distribution of HPV-HR by age and genotypes.

Age groups	Types of infections	HPV-HR Carriage Genotypes (number of women)	Total	
			women	Genotypes
≤19 years	Isolated	52 (1)	1	1
	Multiples	51 + 52 + 68 (1)	1	3
20 - 25 years	Isolated	31 (1); 51 (1); 52 (3); 66 (6); 68 (1); 39 (2);	14	14
	Multiples	18 + 59 (1); 35 + 52 (2); 35 + 66 (1); 39 + 68 (2)	6	12
26 - 30 year	Isolated	18 (1); 31 (1); 52 (1); 58 (2); 59 (1); 66 (2); 68 (2)	10	10
	Multiples	31 + 35 + 51 + 56 + 58 (1); 56 + 66 (1)	2	7
31 - 35 years	Isolated	45 (1); 51 (1); 52 (1); 56 (1); 59 (1)	5	5
36 - 40 years	Isolated	18 (1); 39 (1); 51 (1); 52 (1)	4	4
	Multiples	18 + 39 + 52 (1); 51 + 68 (1)	2	5
41 - 45 years	Isolated	31 (1); 45 (1)	2	2
46 - 50 years	Isolated	56 (1)	1	1
Total			**48**	**64**

3.5. HPV-HR Carriage According to Sexual and Behavioral Characteristics

The data are shown in Table 5. The frequency of HPV-HR distribution was so high than women had a weekly high frequency of sexual intercourse ($p = 0.000$).

4. Discussion

The results of this work cannot be generalized at the national level, let alone subregional, given the size of the sample. However, they constitute a link that can be integrated into other results for more comprehensive information on the different genotypes of HPV-HR circulating in Burkina Faso.

In this work we have described the epidemiology of high-risk human papilloma virus infection at a time when Burkina Faso is preparing to start a vaccination program against this virus. The prevalence of HPV-HR infection was 20.6% in our study. Previous studies in Burkina have reported generally higher rates

Table 5. HPV-HR carriage according to sexual and behavioral characteristics.

Characteristics	HPV-HR		Total	p
	Positive	Negative		
Marital status				
Married	38	160	198	0.2320
Single	10	23	33	
Widowed	0	3	3	
Total	48	186	234	
Age at first intercourse				
<19 years	29	118	147	0.5730
19 à 30 years	19	67	86	
>30 years	0	1	1	
Total	48	186	234	
Weekly frequency of sexual intercourse				
1 to 2	43	153	196	0.000
More than 2	5	33	38	
Total	48	186	234	
Condom use during sex				
Never	39	138	177	0.3740
Sometimes	2	24	26	
Rarely	4	15	19	
Every time	3	9	12	
Total	48	186	234	
History of genital infections				
Yes	20	71	91	
No	28	115	143	
Total	48	186	234	

ranging from 25.4% to 72.6% [3] [4] [5] [6] [7]. Higher prevalences around 30% are also reported internationally [8] [9]. Our result is similar to that reported by Confortini M *et al.* in Italy in 2010 and Ngabo F in Rwanda in 2014, respectively 20% and 22% [10] [11]. HPV infection remains the first STI in the world [12] [13].

In our study, the prevalence of HPV-HR infection was significantly higher among young women. Several authors have reported that this prevalence is inversely proportional to the age of the patients [14] [15] [16].

The prevalence of isolated genotypes varies widely across studies and regions. In the work of Monsenego in the USA, Hibbits in the UK, Pamato in Italy and Mollers in Netherlands HPV 16 is the genotype most frequently found [17] [18] [19] [20]. It is the same for Dols in South Africa [21]. The genotypes most fre-

quently found in our study were HPV 52 and 66 (17.2% and 15.6%) and HPV 16 was not found. The first two genotypes reported in other studies conducted in Burkina Faso are in order of frequency HPV 52, 35, 39, 33, 56, 18, 16 and 66 [3] [4] [5] [6] [7] [22] [23]. Six of these HPV-HR are not covered by the available vaccines in Burkina Faso. In the sub-Saharan subregion, the first two most frequently reported genotypes are 16, 52, 35, 18, 33, 39, 42, 50, 58 and 59 [3] [5] [6] [21] [24] [25].

Overall prevalence of infection as well as multiple infections (2 - 5 genotypes) were higher among young women. The same observation is reported in the literature [4] [5] [18] [26] [27]. All these studies agree that the infection is more important in younger women who have more intense sexual activity, and decreases with age due to the clearance of the virus and the acquisition of some immunity.

Cervical abnormalities detected with IVA/IVL showed no significant association with HPV-HR carriage ($p = 0.8560$).

Some studied risk factors, such as age at first sexual intercourse, use of condom, marital status, level of education, or previous history of genital infection, were not significantly related to the carriage of HPV-HR. However, we found a statistically significant association between the frequency of sexual intercourse and HPV carriage ($p = 0.0000$). Indeed, 73.4% of women with HPV had more than two sexual intercourses a week. Figueroa et al also found a highly significant association between high prevalence of HPV and frequent sexual activity [28].

5. Conclusion

Our results highlight a predominance of HPV-HR genotypes not covered by commercially available vaccines. Genotypes mapping at the national level, even sub-regional, seems necessary for us to understand our viral ecology. This would, as a first step, better guide the choice of vaccines to use for our populations; and in the next steps, serve as a basis for the development of multivalent vaccines better adapted to circulating viruses in our resource-limited countries. In the immediate future, the nonavalent vaccine, which has a better coverage of the predominant genotypes, is to be promoted.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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