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HPV/HBV or HPV/HCV Co-Infections in Women Treated for Chronic Hepatitis at Hôpital Saint Camille in Ouagadougou, Burkina Faso

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Abstract

Introduction: Cervical cancer is a public health concern and is mainly caused by Human papillomaviruses (HPV). In many parts of the world, studies are being carried out to understand the different genotypes to better tackle this issue. We conducted a study to determine the prevalence of HPV genotypes in women with chronic hepatitis B or C infection, co-infected or not with HIV, treated at the Hôpital Saint Camille in Ouagadougou (Burkina Faso). **Methods:** This study was conducted from April to July 2023, including 100 women in gastroenterology at Hôpital Saint Camille. A questionnaire on their socio-demographic and life style was administrated; and endocervical samples were collected using sterile swabs and then sent to Centre of Biomolecular Research Pietro Annigoni (CERBA). HPV molecular detection and genotyping were performed by PCR and hybridization using the HPV Direct Flow Chips kit. Data were analysis using chi square test or Fischer's exact test with

a significance threshold for $p < 0.05$. **Results:** The prevalence of HPV infection was 28% (28/100) on the sample of women tested. The most frequent genotypes were HPV 52 (8.33%), followed by HPV 18 and 68 (6.25% each) for high-risk HPVs, and HPV 6, 44/55 and 62/81 (8.33% each) for low-risk HPVs. **Conclusion:** This study, the first of its kind in Burkina Faso on this group of the population, reveals that the most frequent genotypes found in this study are not included in the vaccine available in Burkina Faso (Gardasil®4).

Keywords

Human Papillomavirus, Cervical Cancer, Hepatitis, Human Immunodeficiency Virus, Burkina Faso

1. Introduction

Cervical cancer is a major global public health problem, with 604,000 new cases and 342,000 deaths worldwide in 2020 [1]. It is much more widespread and devastating in developing countries [2] and linked to persistent infection with the Human Papillomavirus (HPV) [3]. This sexually-transmitted infection is widespread in the general population. It is the most common viral infection of the reproductive tract, with over than 100 different types of HPV identified [3] [4]. Some HPV genotypes can cause genital warts known as low-risk HPV (LR-HPV), while others can lead to the development of various types of cancer, notably of the cervix (high-risk oncogenic HPV or HR-HPV) [5].

In Burkina Faso, cervical cancer is the second most common cancer in women, with 1132 cases and 839 deaths estimated for 2020 [6]. Several studies have already been carried out to determine the prevalence of HPV infection in the general population in different regions and towns of the country [7] [8] [9] [10], among sex workers [11] [12], among women diagnosed with precancerous lesions [13] [14] [15], in those with invasive cervical cancer [16] [17], in pregnant women [18], in adolescents [19] and in people living with Human Immunodeficiency Virus (HIV) [20] [21] [22]. However, no studies have been carried out in women with hepatitis B (HBV) and hepatitis C (HCV) infection. Yet hepatitis B and C are another public health concern, and can also cause hepatocellular carcinoma, another type of cancer. Hepatitis B and C viruses are also transmitted sexually, and together account for three-quarters of hepatocellular carcinomas [23]. Previous study from Burkina Faso had reported a prevalence of 9.1% and 3.6% for HBV and HCV respectively [24]. Another study from Burkina Faso had reported a prevalence of 14.5% and 1% for HBV and HCV respectively [25]. Patients already suffering from hepatitis B and C have a weak immune system and are more likely to carry out HR-HPV or other viral infections. In addition, the coexistence of several viruses in one's body may promote the progression to

cancer. HPV/HBV or HPV/HCV co-infections could therefore increase the risk of cancer, particularly when associated with HIV infection. Moreover, several epidemiological studies have suggested a significant association between HBV and HCV infection and the risk of extrahepatic cancers. Indeed, the presence of HBV in vaginal fluids makes it biologically plausible that HBV infects the cervical epithelium. Some authors hypothesized that HBV may interact with HPV to induce and promote the pathogenesis of cervical cancer [26].

We conducted the present study to determine the prevalence of HPV infection and the different genotypes in women infected with HBV or HCV, with or without HIV co-infection threated at the Hôpital Saint Camille in Ouagadougou (Burkina Faso).

2. Material and Methods

2.1. Study Population

This study was conducted from April to July 2023. One hundred (100) women undergoing treatment for chronic hepatitis B or C at Hôpital Saint Camille de Ouagadougou (HOSCO) who had given their free and informed consent constituted the study population. Samples for HPV testing were swabbed from women endocervix using a sterile cotton-tipped swab and sent to the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA, Ouagadougou) for HPV detection.

Women attending gastroenterology consultations and suffering from chronic hepatitis B or C during the study period were approached individually. We explained the role of HPV infection in the development of cervical cancer, the risks factors. We also explained the benefits of regular screening and the aim of the study. They were then given the opportunity to ask questions to gain a better understanding. Those who agreed and met the inclusion criteria were then included in the study.

Virgins, menstruating women, women who had undergone a hysterectomy and women who had not given their consent were excluded from the study. A questionnaire was administered to each woman to ascertain their socio-demographic characteristics (age, education level, profession…) and lifestyle (age at first intercourse, number of sexual partners…).

2.2. Ethical Considerations

This study was authorized by the institutional ethics committee of Hôpital Saint Camille de Ouagadougou in February 12, 2023, deliberation No. 2023-03-012.

2.3. HPV Detection

Endocervical samples were tested for HPV genotypes by Polymerase Chain Reaction (PCR)/hybridization using the HPV Direct Flow Chip kit (Vitro Master Diagnóstica). This method, based on PCR followed by hybridization, identi-

fied 36 HPV genotypes, including 18 high-risk genotypes (HR-HPV) such as HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82, and 18 low-risk genotypes (LR-HPV), namely HPV 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84 and 89. Samples were pretreated twice with DNAse/RNAse free solution and 30 µL of each sample was then added to the PCR mix. PCR was performed on GeneAmp PCR System 9700 (Applied Biosystems) according to the following amplification program: 1 cycle (25˚C for 10 minutes); 1 cycle (94˚C for 3 minutes); 15 cycles (94˚C for 30 seconds, 47˚C for 30 seconds, 72˚C for 30 seconds); 35 cycles (94˚C for 30 seconds, 65˚C for 30 seconds, 72˚C for 30 seconds) and 1 cycle (72˚C for 5 minutes). The PCR products obtained were then denatured at 95˚C for 10 minutes, and semi-automated hybridization was carried out using HybriSpot12 (Vitro Master Diagnóstica). Image capture, analysis and reporting of results were carried out using HybriSoft software.

2.4. Data Analysis

All variables were categorical and were presented as percentages. The Chi-squared test (or Fischer's exact test when values were less than 5) was used to compare the sociodemographic characteristics between women positive and those negative for HPV. The difference was considered statistically significant for $p < 0.05$. Data were processed and analyzed on the computer using R, Excel 2016, and Epi Info 7.2.5.0 software.

3. Results

3.1. Patients Characteristics

A total of 100 samples were collected and used for HPV genotyping. Patient characteristics are presented in Table 1. They had an average age of 37.8 ± 11.02 years (range 20 - 65 years) and a median age of 37 years (95% CI: 35.7 - 40.0). The most representative age group was 30 - 39 with a percentage of 39% (39/100). Married women and those living with a partner represented 72% (72/100), and 70% (70/100) of the female study population respectively. Women working in the informal sector and housewives were the most represented, with percentages of 36 and 29 respectively. Age at first intercourse ranged from 14 to 30 years, with an average of 18.8 ± 2.1. Women who had had only one sexual partner in their lifetime numbered respectively 40 (40%), and 21 out of 100 women had never had a pregnancy. Only 11% (11/100) were using oral contraception at the time of the study, with the majority using no method at all. In addition, 3% (3/100) and 4% (4/100) of the women were HIV-positive and unknown respectively. Of the 100 women, 99 had hepatitis B and only one had hepatitis C. There were no cases of hepatitis B/C co-infection. More than half the women (58/100) had already been screened for cervical cancer in the last 5 years, with negative results. Of these 58 women, two (2) had already had an HPV test, which was negative, and were therefore informed about HPV and its consequences.

3.2. Prevalence of HPV Infection

In this study, we determined the prevalence of both HR-HPV and LR-HPV in 100 Burkinabè women infected with HBV or HCV. Overall HPV prevalence (HR-HPV and LR-HPV) was 28% (28/100; CI 95%: 19.20 - 36.80), including 2 women with indeterminate genotypes. Among the 26 HPV-positive women whose genotypes could be determined, we were able to identify twenty-five (25) different HPV genotypes, including 14 high-risk HPVs and 11 low-risk HPVs at different frequencies (for a total of 48 genotypes). A total of 18 women had at least one HR-HPV (18/100) and 21 (21/100) had at least one LR-HPV. Isolated infections were 46.43% (13/28) versus 53.57% (15/28) for multiple infections. The total number of genotypes per infected woman ranged from 1 to 4. Of the high-risk HPVs, the three (3) most frequent were HPV 52 (4/48; 8.33%) followed by HPV 18 and 68 (3/48; 6.25% each).

Among low-risk HPVs, HPV 6, 44/55 and 62/81 were the 3 most frequent, with a percentage of 8.33% each (4/48) (Figure 1).

The three (3) HIV positive women were on Antiretroviral Therapy (ART) and were all HPV-positive, with several genotypes ranging from 2 to 4 HPV types.

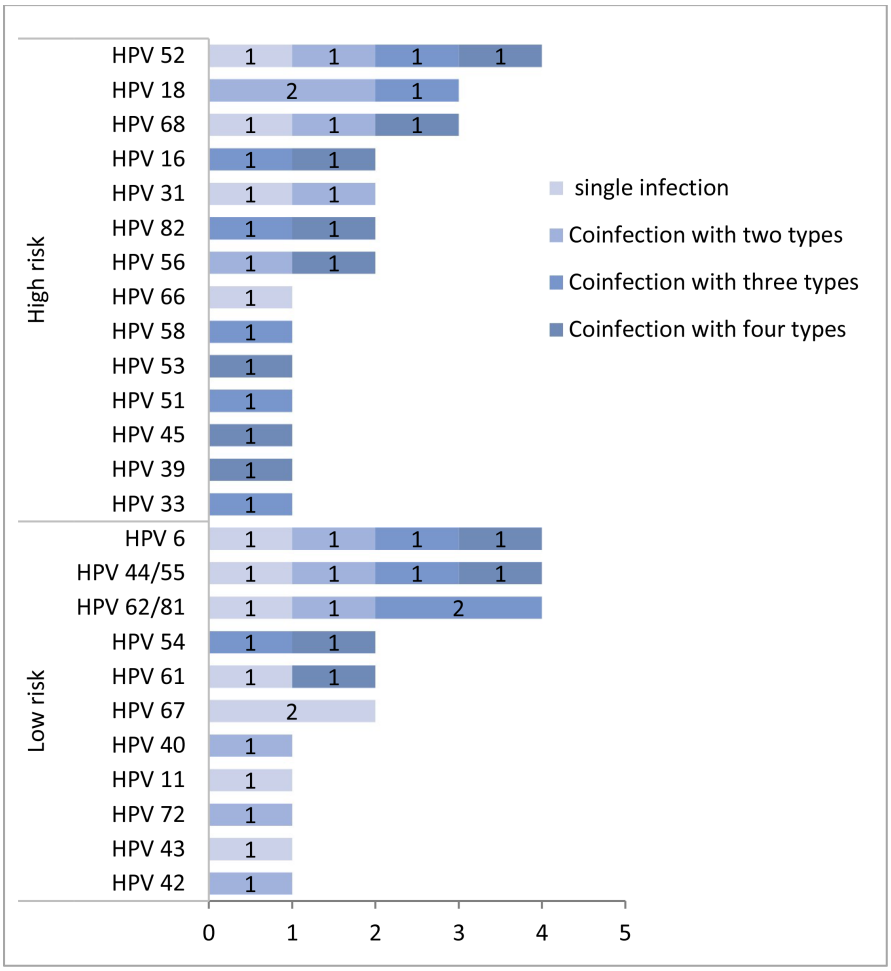

Figure 1. HPV genotypes in women treated for chronic hepatitis B or C in Burkina Faso.

They were infected with both high-risk and low-risk HPV. We also analyzed the association between HPV infection and age, age at first sexual intercourse, educational level, marital status, number of sexual partners, occupation, parity, use of oral contraception, cervical screening and HIV status using the chi-squared test (Table 1). However, only educational level, parity and HIV status was found to be significantly associated with the carriage of HPV infection (with p-value of 0.010, 0.006 and 0.002 respectively).

Table 1. Socio-demographic and behavioral characteristics of HBV/HCV-positive women according to HPV status.

Characteristics	Frequency (%) N = 100	HPV status		P-value
		HPV+ % (N = 28)	HPV− % (N = 72)	
Age (years)				
20 - 29	24	10	14	
30 - 39	39	11	28	0.311
40 - 49	19	4	15	
50 - 65	18	3	15	
Education level				
None	13	0	13	
Primary	17	3	14	0.010*
Secondary	44	19	25	
University	26	6	20	
Profession				
Pupils/students	12	2	10	
Housewives	29	9	20	0.787
Informal sector	36	11	25	
Civil servants	23	6	17	
Marital status				
Single	18	8	10	0.128
Married/Concubine	72	19	53	
Widow	10	1	9	
Age at first intercourse (years)				
<18	23	10	13	0.169
18 - 24	60	14	46	
25 - 30	17	4	13	
Number of sexual partners since first intercourse				
1	40	10	30	0.563
2 - 4	56	16	40	
≥5	4	2	2	

Continued

Parity				
0	21	10	11	
1 - 2	38	14	24	0.006*
3 - 5	33	3	30	
5 - 8	8	1	7	
HIV status				
Negative	93	22	71	0.002*
Positive	3	3	0	
Unknown	4	3	1	
Type of hepatitis				
B	99	28	71	1
C	1	0	1	
Oral Contraception				
Yes	11	3	8	>0.999
No	89	25	64	
Previous screening for cervical cancer				
Yes	58	17	41	0.907
No	42	11	31	

*P-value is significant.

4. Discussion

We conducted this study on 100 women infected with VHB or VHC to research the prevalence of HPV and the different genotypes in this population. Only one woman in the study population was chronically infected with hepatitis C. This could be explained by the lower prevalence of hepatitis C in the general population as found in previous studies. Indeed, previous research reported a hepatitis C prevalence of 1% in the general population and 1.47% in women [25] [27] in Burkina Faso. In addition, prevention of hepatitis B is much more widespread through screening-vaccination campaigns, and also during pregnancy among women, compared with hepatitis C. In Brazil, a similar study on 103 people (45 men and 58 women) obtained 48 infected with hepatitis B, 55 with hepatitis C and 2 co-infected with hepatitis B/C [28]. This difference may be explained by the fact that this study included both men and women. The only female hepatitis C patient in our study was found to be HPV-negative. This does not, therefore, allow us to speculate on the possibility that hepatitis C virus may play a role in HPV infection.

Of the 96 women who knew their HIV status, 3 were HIV-positive and on antiretroviral therapy. All were HPV-positive, with several genotypes ranging from 2 to 4 HPV types. They were infected with both high-risk and low-risk HPV. HIV has already been documented as a risk factor for HPV infection and persis-

tence, and for multiple HPV types, even when treated with ART [29] [30] [31]. Two studies in Burkina Faso found prevalences of 58.33% and 59.5% respectively of HPV infection in HIV-positive women [20] [22]. In addition to HIV, educational level and parity was significantly associated with HPV carriage. Other studies reported educational level and parity as risk factors for HPV carriage [32] [33]. Educational level has been reported as cofactor for risk of cervical cancer. Women with a low level of education tend to have more sexual partners, are less likely to be screened and have a higher prevalence of HPV [34]. The increased levels of oestrogen and progesterone during pregnancy possibly induce a reduced immune response to HPV infection and influence the risk of persistence or progression to cervical cancer [35].

HPV molecular testing has been widely used in many studies as a primary screening test for cervical cancer [36] [37] [38]. The 28% prevalence of HPV-positive women we found, although high, is lower than the 35.40% prevalence obtained among women of childbearing age in Burkina Faso by a previous study [7]. This could be explained by the much larger sample size (1321) and could mean that viral hepatitis B and C do not play a role in the occurrence or persistence of HPV infection. The prevalence found in our study was higher than that found among pregnant women (23%) in Burkina Faso with the same sample size as ours [18]. The Brazilian study found 20/103 HPV-positive individuals, with the 2 hepatitis B/C coinfected individuals being HPV- and HIV-positive [28]. Thus, while chronic hepatitis B or C infection does not seem to correlate with the acquisition of HPV infection, it is clear that HBV/HCV/HIV co-infection is a risk factor for HPV carriage.

Among the 28 HPV-positive women, genotypes could not be determined for 2 women. Among the 26 whose genotypes were determined, we found 25 different genotypes, for a total of 48 genotypes. 21 of the 26 women had at least one type of low-risk HPV, sometimes associated with multiple infections of high-risk HPV. Although low-risk HPVs do not cause cervical cancer, the benign lesions for which they are responsible cause stress and discomfort in patients, particularly HPV 6 and 11. In this study, the most common low-risk HPV types were HPV 6, 62/81 and 44/55. HPV 11 was found in only one patient. In pregnant women, [18] found HPV 6, 62/81 and 44/55 with respective percentages of 3.45%, 6.89% but HPV 11was not found. We found 53.57% multiple infections in this study. Infection with multiple HPV genotypes is associated with an increased risk of HPV persistence and progression to precancerous and cancerous cervical lesions [39]. The most common high-risk genotype was HPV 52. Previous data showed a higher HPV 52 prevalence of 10.9% in Burkina Faso and Côte d'Ivoire [40], 10.34% in Burkina among pregnant women [18], 10.68% in Burkina, Benin, Côte d'Ivoire and Niger [41].

The Gardasil 4 vaccine, comprising virus-like particles of types 6, 11, 16 and 18, has recently been introduced in Burkina Faso, with the potential to prevent cervical cancer, although it is not yet available to the entire Burkinabè popula-

tion. All 4 genotypes were found in our study. However, they were not the most frequent genotypes. It would therefore make more sense to introduce a vaccine including the most frequent genotypes, such as 52.

5. Conclusion

HPV is the cause of cervical cancer. They are widespread in the general population, including women with chronic hepatitis B or C infection. This segment of the female population is vulnerable, especially when co-infected with HIV. The relatively high prevalence of HPV infection among these women calls for further investigation with a more representative sample to better guide HPV and cervical cancer vaccination policy in Burkina Faso. In addition, the introduction of gardasil 9 vaccine by government would enable wider coverage, given the genotypes circulating in this segment of the population.

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Conflicts of Interest

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

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