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Genotypic Distribution of the Human Papillomavirus among Women with Cervical Cytological Abnormalities at the Sourô SANOU University Hospital in Bobo-Dioulasso, Burkina Faso

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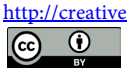
Abstract

Cervical cancer is the fourth most common cancer worldwide, accounting for 6.8% of new cancer cases and 8.1% of cancer-related deaths. About 85% of these deaths occurred in low- and middle-income countries. The aim of this study was to assess the frequency and distribution of the human papillomavirus (HPV) genotypes in women showing cytological abnormalities of the cervix at the Sourô SANOU University Hospital (CHUSS) in Bobo-Dioulasso, Burkina Faso. This is a descriptive study of women recruited at the CHUSS. The cervico-uterine smear examination was carried out at the CHUSS Anatomy and Pathology Department for cervical cancer screening. The data were collected from women with atypical cells on their cervico-uterine smear. Cervicovaginal samples were taken from consenting women and HPV genotyping was performed using the HPV Direct FLOW CHIP kit at CERBA. We obtained approval from the ethics committee. The data were analyzed using the SPSS 26 software. The results of the study showed that 67.79% of the participants were aged between 50 and 65, a group that is particularly vulnerable to persistent infection with high-risk oncogenic HPV genotypes. Of the women



screened, 40.7% were HPV positive and 29.2% had multiple infections. The most common genotypes were HPV 35, followed by HPV 18, 52, 58 and 66. These data highlight the need for increased surveillance and targeted prevention strategies among this female population.

Keywords

Genotyping, Human Papillomavirus, Cervical Cytological Abnormalities, Burkina Faso

1. Introduction

Cervical cancer is the fourth most common cancer in the world, accounting for 6.8% of new cancer cases and 8.1% of cancer-related deaths worldwide [1]. About 85% of these deaths occurred in low- and middle-income countries [2]. According to GLOBOCAN, in Burkina Faso, it remains the second most common cancer in women after breast cancer and this country recorded 988 new cases with 775 deaths in 2022. Cervical cancer is a major public health problem worldwide, particularly in low-income countries where the incidence is highest. Women in sub-Saharan Africa have the highest prevalence [3], which appears to be increasing over time [4]. High-risk human papillomaviruses (HPV) are responsible for around 99% of cervical cancer cases [5]. It is the most common sexually transmitted infection in the world [5]. These HPVs produce viral oncoproteins which, when produced over a long term, disrupt cell cycle control, leading to the development of cervical intraepithelial neoplasia (CIN), precursor to cervical cancer [6]. According to the World Health Organization (WHO), cervical cancer is preventable through vaccination, screening and treatment of precancerous lesions [7]. Although high-risk papillomavirus infection might occur, that alone is not enough to develop into cancer as most infections are latent and regress spontaneously without treatment [8]. However, other factors, such as high parity and poor socio-economic conditions, also contribute to the development of cervical dysplasia [9]. Most precancerous lesions and cancers of the cervix, on the other hand, are associated with infection with high-risk, oncogenic high-risk human papillomavirus infections [10]. Cervical cancer takes around fifteen to twenty years to develop after persistent high-risk HPV infections [11]. The onset of cancer requires the passage of precancerous lesions, leaving an important window of opportunity for cancer prevention. The slow development of precancerous lesions means that they can be detected before they reach the invasive stage. Precancerous lesions can be detected through cytology, which classifies abnormalities according to the Bethesda 2001 terminology, visual inspection with acid and Lugol's iodine (VIA/VILI) [12] and HPV testing [13]. The aim of this study is to assess the prevalence and distribution of HPV genotypes and the epidemiological aspects of women showing abnormal cells in cervical smear tests, in order to help improve patient management.

2. Methods

2.1. Scope and Type of Study

This research study took place in Burkina Faso, more specifically in Bobo Dioulasso, the country's economic capital. This was a descriptive study with an analytical purpose. The women were recruited at the Sourô SANOU University Hospital (CHUSS). The cervico-uterine smear test was performed at the Cytology, Anatomy and Pathology department and the HPV genotyping was performed at the HPV National Reference Laboratory (HPV-NRL) of Pietro ANNIGONI Biomolecular Research Centre (CERBA/LABIOGENE), in Burkina Faso.

2.2. Study Population and Inclusion Criteria

The population of this study were women screened for cervical cancer with cervical smear for the presence of atypical or abnormal cells.

Inclusion criteria: All patients with atypical cells on the cervico-uterine smear at the time of pathological diagnosis and who gave their consent.

Non-inclusion criteria: Women who had undergone total hysterectomy and women who did not consent to taking part in the study.

2.3. Data Collection

Socio-demographic data and women's knowledge, attitudes and practices were collected using a questionnaire integrated into the KoboToolbox application.

2.3.1. Cervico-Uterine Smear Test to Screen for Precancerous Lesions

The pathologist spread a thin layer in a regular, linear and continuous shape on two slides. Samples were taken from the ectocervix and the ectocervical junction using the rounded end of the Ayre spatula, which has a special shape that makes it possible to scrape elements from the endovaginal part of the ectocervix, particularly to obtain cells from the junction zone between the squamous epithelium of the ectocervix and the glandular epithelium of the endocervix, the birthplace of cervical dysplasia.

2.3.2. Cervicovaginal Sampling for Human Papillomavirus Genotyping

After exposing the cervix with a speculum, a cotton-tipped swab is inserted into the endocervix and the ectocervix at the junction and rotated clockwise at least three times. The collected sample is then placed in a 1.5 mL extraction tube and placed at −80˚C pending HPV genotyping.

2.4. Screening for Precancerous Lesions Using the Papanicolaou Test

The smears were stained by the Papanicolaou method as follows:

The smears were fixed with CitoSpray solution and then left to dry. The slides were then immersed in 70˚ alcohol for 10 seconds and in 50˚ alcohol for 10 seconds, then rinsed with distilled water. The slides were immersed in Harris haematoxylin for 2 min 15 seconds, then rinsed with distilled water. The smears were stained with 70˚ alcohol for 20 seconds, 80˚ alcohol for 10 seconds, 95˚ alcohol

for 10 seconds and then immersed in the OG6 solution for 1 min 30 seconds. In addition, they were run in 95˚ alcohol for 20 seconds, in EA50 solution for 01 min 45 seconds, then in 95˚ alcohol for 20 seconds, in 100˚ alcohol for 20 seconds, in xylene for 20 seconds and finally in Eukitt. The smears were assessed, and the microscopic reading results were provided by CHUSS pathologists.

2.5. HPV Genotyping

Molecular characterization of HPV was performed by PCR/hybridization using the HPV Direct Flow Chip kit (Vitro Master Diagnóstica) according to the manufacturer's protocol [14]. Multiplex PCR was performed using the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) and hybridization was performed using the semi-automatic HybriSpot 12.

2.6. Ethical Considerations

The protocol was approved by the Ethics Committee for Health Research (CERS) during deliberation referenced 2023-07-182. Authorization was obtained from the CHUSS Senior Management Staff and the participants' free and informed consent was obtained before all data were collected. The confidentiality and anonymity were respected and information obtained from the various patient's histories were kept confidential.

2.7. Data Analysis

Data were extracted from the server in Excel format and processed by the IBM Statistical Package for Social Sciences (SPSS) software version 26. Chi-square test was used for comparisons, with 95% confidence interval. The difference was statistically significant at $p < 0.05$.

3. Results

3.1. Socio-Demographic Characteristics of the Study Population

Table 1 presents the socio-demographic characteristics selected for this study. It provides an overview of the participants' attributes, which are essential for understanding the context of the study. The majority of women were married (57.62%) with an average age of 54.71 ± 10.28 years. The ages of the women ranged from 29 to 78, and were mainly concentrated around the ages of 50 and 65. In addition, the level of education was relatively high. Over 72% had at least primary education. However, over 50% were housewives, and women with no education at all, accounted for 13.5%.

3.2. Behavioral and Sexual Characteristics and Precancerous Lesions of the Women Included in the Study

Table 2 provides crucial data for understanding the risk factors associated with HPV infections in the study population. Age at first intercourse varied between 15 and 29. More than half of the women (84.74%) had their first sexual intercourse

between the ages of 15 and 19, and most (55.93%) said they had only one sexual partner. All the women had been pregnant before, over 40% of whom had had at least 5 confirmed pregnancies, while 3.3% were nulliparous. Almost all the women (61%) did not use condoms during sexual intercourse. Less than 20% of the women had abortions before. The HIV status of 74.58% of women was negative and 8.48% of the participants did not know their HIV status. However, 16.94% tested positive and are all on antiretrovirals (ARVs). The only women whose cervical smear revealed the presence of abnormal cells were recruited for this study. More than half of the population studied (55.9%) had Low Grade Squamous Intraepithelial Lesion (LGSIL), 35.6% had Atypical Squamous Cells of Undetermined Significance (ASCUS) and 8.5% had High Grade Squamous Interepithelial Lesion (HGSIL).

Table 1. Socio-demographic characteristics and risk factors associated with HPV infection and the development of precancerous lesions.

Characteristics	Frequency n (%)	HPV status		p-value	Cytological abnormalities			p-value
		Positive	Negative		HGSIL	LGSIL	ASCUS	
Age (Years)								
20 - 34	3 (5.08)	2	1	0.24	0	2	1	0.49
35 - 49	11 (18.64)	4	7		0	7	4	
50 - 65	40 (67.80)	17	23		4	22	14	
>65	5 (8.47)	1	4		1	2	2	
Level of Education								
None	8 (13.56)	2	6	0.54	2	4	2	0.77
Primary	18 (30.51)	9	9		1	10	7	
Secondary	25 (42.37)	11	14		2	15	8	
Koranic school	8 (13.56)	2	6		0	4	4	
Marital status								
Single	3 (5.08)	3	0		0	3	0	
Married	34 (57.62)	10	24		1	18	15	
Widow	19 (32.20)	9	10		3	11	5	0.08
Divorce	2 (3.38)	1	1		0	1	1	
Free union	1 (1.69)	1	0		1	0	0	
Profession								
Housewife	40 (67.80)	14	26	0.17	4	20	16	0.49
Shop keeper	7 (11.86)	2	5		0	5	2	
Salary earner	12 (20.34)	8	4		1	2	3	

Table 2. Behavioral and sexual characteristics and risk factors associated with HPV infection and the development of precancerous lesions.

Characteristics	Frequency n (%)	Precancerous lesions			Total	p-value	HPV Status		p-value
		HGSIL (n)	LGSIL (n)	ASCUS (n)			HPV⁻ (n)	HPV⁺ (n)	
		5	33	21	59		24	35	
Age at first sex									
15 - 19	50 (87.75)	4	28	18	50		29	21	
20 - 24	7 (11.86)	0	5	2	7	**0.012**	5	2	0.93
>24	2 (3.39)	1	0	1	2		1	1	
Number of sexual partners									
0	1 (1.67)	0	1	0	1		0	1	
1	33 (55.93)	4	17	12	33		20	13	
2	10 (16.95)	1	4	5	10	0.76	7	3	0.68
3	7 (11.86)	0	5	2	7		4	3	
4	8 (13.56)	0	6	2	8		4	4	
Condom use									
Yes	3 (5.08)	1	1	1	3		0	3	
No	36 (61.02)	1	23	12	36		24	12	
Sometimes	6 (10.17)	1	2	3	6	0.77	3	3	0.24
Don't know	9 (15.25)	1	3	5	9		5	4	
No answer	5 (8.48)	1	4	0	5		3	2	
Pregnancy									
1	6 (10.17)	0	6	0	6		1	5	
2	2 (3.39)	1	1	0	2		1	1	
3	8 (13.56)	1	3	4	8	0.36	4	4	0.20
4	8 (13.56)	1	3	4	8		5	3	
5	9 (15.25)	0	4	5	9		6	3	
>5	26 (44.07)	3	14	9	26		18	8	
Parity									
0	2 (3.39)	0	2	0	2		0	2	
1	4 (6.78)	0	4	0	4	0.51	1	3	**0.032**
2	6 (10.17)	1	4	1	6		1	5	
3	13 (22.03)	1	8	4	13		9	4	

Continued

4	9 (15.25)	0	3	6	9		8	1	
5	9 (15.25)	0	5	4	9	0.51	4	5	**0.032**
>5	16 (27.12)	3	7	6	16		12	4	
Number of abortions									
0	48 (81.35)	5	25	18	48		30	18	
1	7 (11.86)	0	4	3	7	0.00	4	3	0.62
2	2 (3.39)	0	2	0	2		1	1	
3	1 (1.70)	0	1	0	1		0	1	
HIV status									
Positive	10 (16.95)	0	9	1	10		4	6	
Negative	44 (74.58)	4	22	18	44	0.31	30	14	**0.045**
I don't know	5 (8.47)	1	2	2	5		1	4	
HPV status									
Negative	24 (40.68)	2	20	13	24	0.48			
Positive	35 (59.32)	3	13	8	35				

Legend: LGSIL: Low Grade Squamous Interepithelial Lesion; HGSIL: High Grade Squamous Interepithelial Lesion; ASC-US: Atypical Squamous Cells of Undetermined Significance.

3.3. Risk Factors Associated with HPV Infection and the Development of Cervical Cancer

In this study, women who had their first sexual intercourse between the ages of 15 and 19 had more precancerous lesions, the majority of which were high-grade lesions. This difference was statistically significant (p-value = 0.012), while socio-demographic characteristics such as age, education, marital status and occupation were not associated with HPV status and precancerous lesions in this study. HIV status was associated with persistent HPV infection (p-value = 0.045). The number of confirmed deliveries was associated with HPV infection (p-value = 0.032). Similar to these risk factors, behavioral and sexual characteristics such as HPV status, number of abortions, parity, number of pregnancies, condom use, and number of sexual partners were not associated with cervical cytological abnormalities.

3.4. HPV Prevalence and Genotypic Distribution in Infected Women

In this study, less than half, 24 women or 40.7% out of 59 women screened were HPV positive. Among them, the genotypes of 21 women were known and 3 with undetermined genotypes. The prevalence of high-risk HPV among infected women was 17 out of 24 or 70%. The HPV 35 genotype was more representative, with a frequency of 8.47%. HPV 18, 52, 58, 66 all had a frequency of 3.39 and HPV

16, 39, 56 and 66 each had a frequency equal to 1.69%. In addition to the high oncogenic risk genotypes found, the prevalence of low oncogenic risk genotypes was 58.34% among those infected, with the most representative being HPV 62/81 followed by HPV42 and 44/45 (Figure 1).

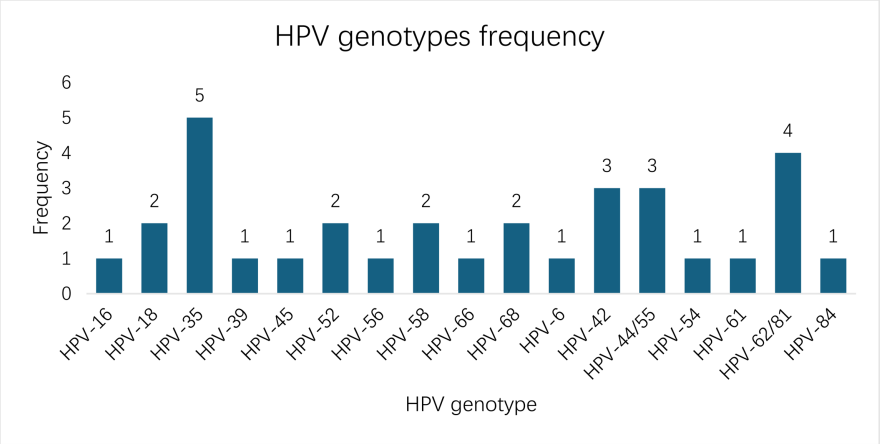


Figure 1. Frequency of HPV genotypes in infected women.

Among the 35 high-risk and low-risk genotypes, 16 genotypes were found including 9 high-risk genotypes (HR-HPV) and 7 low-risk genotypes (LR-HPV). The genotypes found were HPV16/18/35/39/52/56/58/66/68 for those at high oncogenic risks and HPV6/42/44/45/54/61/62/81/84 for those at low oncogenic risks. The number of genotypes found per woman varied between 1 and 4 and multiple infections represented 29.27% of those infected with three (03) undetermined genotypes (Table 3). The HPV prevalence in the cytological abnormality stages was 60%, 39.4% and 38.1% respectively in HGSIL, LGSIL and ASC-US.

Table 3. Distribution of HPV genotypes in multiple infections.

Isolated infection	Frequency	Multiple infections	Frequency
HPV-35	1	HPV35, HPV54	1
HPV-18	1	HPV42, HPV62/81	1
HPV-39	1	HPV56, HPV42	1
HPV-45	1	HPV52, HPV66	1
HPV-58	2	HPV16, HPV18, HPV35	1
HPV-68	2	HPV35, HPV52, HPV62/81	1
HPV-6	1	HPV35, HPV42, HPV44/55, HPV61	1
HPV-44/55	2	**Total 2**	**7**
HPV-62/81	2		
HPV-84	1		

Continued

Genotype not determined	3	
Total 1	17	
	Total 1 and 2	24 (40.68)

3.5. Vaccination Coverage According to the Genotypes Found

Of the 33 genotypes identified in the studies, only 12.5% were covered by the quadrivalent Gardasil®4 vaccine available in Burkina Faso, 28.13% were covered by the nanovalent Gardasil®9 vaccine and 71.87% were not covered by an HPV vaccine (Figure 2).

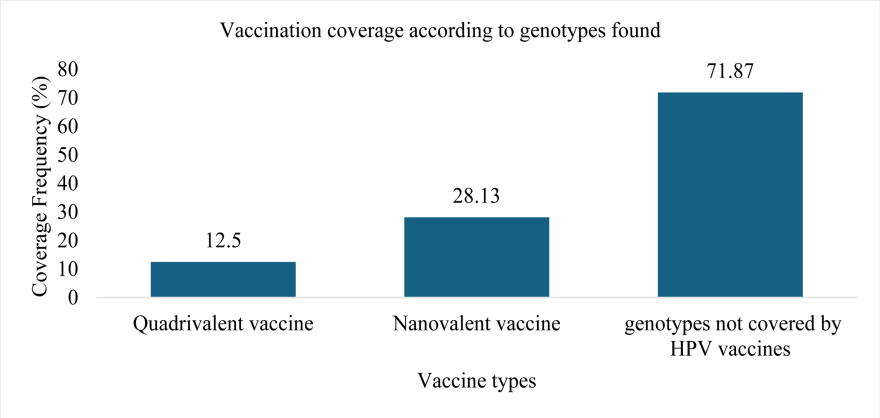


Figure 2. HPV vaccination coverage according to genotype in women.

4. Discussion

Cervical cancer is a multi-stage disease and persistent HPV-HR infection is the main cause of abnormal cytological changes leading to intraepithelial neoplasia and cervical cancer. In low-income countries, screening for precancerous lesions using VIA/VILI is recommended by the WHO because of its affordability and expected results [7]. An effective secondary prevention strategy is to screen suspected cases on visual inspection with acid and Lugol's with a cervical smear to assess cervical cytology. For this reason, the distribution of HPV genotypes in the various cervical lesions remains essential for appropriate management.

The aim of this study was to assess the prevalence and distribution of HPV genotypes and epidemiological aspects of women showing abnormal cells on cervical smears in order to contribute to improving patients' management.

The age of women with atypical cells ranged from 29 to 78 years, and was mainly concentrated around 50 and 65 years, with a mean age of 54.71 ± 10.28 years. Our results were closer to those of other authors such as Zhao *et al.* who reported in their study a mean age of 47.3 ± 7.8 years in a rural Chinese population [15] and different from the results of Ma *et al.* who reported a mean age of 32.52 ± 7.77 years [16]. This difference could be explained by the profile of our study population. Contrary to the results of Zhao *et al.*, more than 75% of the women in

this study were postmenopausal and married. The age distribution of the frequency of cervical cytological abnormalities showed that the 50 to 65 age group was the most affected. This age group corresponds to the menopause. Menopausal hormones fluctuate significantly as a result of the decline in ovarian function. As a result, the immune response of human physiological properties diminishes with age, and the ability of immune systems to eliminate and inhibit the virus diminishes. In addition, the susceptibility to form a persistent infection or to activate the virus during the incubation period has been increased, and the risk of forming a multiple infection is higher [17]. Kabibou *et al.* indicated that the 21 to 25 age group was the most affected by condylomas and the 31 to 35 age group by CIN [18] and Rhouma *et al.* found that the population under 50 was the most vulnerable to the development of precancerous lesions [19]. Our results are consistent with those of Zhao *et al.* who reported that precancerous lesions were more frequent in women over 47 years of age. These differences in results could be explained by the type of study populations and the nature of the samples in the other studies, which were biopsy samples. A number of socio-demographic, behavioral and sexual characteristics contribute to an infection and the development of precancerous lesions or cytological abnormalities of the cervix. They are considered to be risk factors for HPV infection, which if persist, contributes to the development of precancerous lesions and cervical cancer. In this study, age at first intercourse was statistically associated with HPV infection. HIV status and parity were statistically correlated with the development of precancerous lesions. These results are similar to several studies carried out outside Burkina Faso [18] [20]-[22] and in Burkina Faso [23] [24], hence the importance of raising awareness among the target population.

The infection and persistence of papillomaviruses, particularly those with a high oncogenic risk, remain the main cause of the development of precancerous lesions and cervical cancer. In Burkina Faso, studies have highlighted the prevalence of HPV in precancerous lesions [10] [23] [25]. These HPV prevalences varied according to the studies and Zohoncon *et al.* and Traore *et al.* found an involvement of HR-HPV in the development of lesions with a prevalence of 72.31% and 87.2% respectively. The prevalence of HPV in our study was significantly lower than in these two studies. We did not find a statistically significant correlation between HPV infection and cytological abnormalities. These differences in results could be explained by the size of our sample or the high rate of ASCUS in our study population. Nevertheless, our results corroborate those of Ouédraogo *et al.* in Burkina Faso and Wang *et al.* in China, who found a prevalence of 48.8% and 53% respectively. We observed an upward trend in the overall prevalence of HPV infection alongside an increase in the degree of cervical cytological abnormalities (ASC-US: 38.1%, LIEM-BG: 39.4%, LIEM-HG: 60%). Wang *et al.* had reported this increasing trend, but the prevalence of HPV in H-SIL was greater than 80%. This was also reported by Guan *et al.* who found an overall HPV prevalence of 84% and 85% in LIEM-HG cases in Europe and worldwide, respectively [26]. In a systematic review of the literature in

Burkina, Zohocon *et al.* found a prevalence of 69.23%, including cases of precancerous lesions and cases of cancer [20].

In Burkina Faso, several epidemiological studies on HPV in sexually active women have reported a lower prevalence of HPV than in the present study [14] [27]-[29]. Our results suggest that the government of Burkina Faso in general and particularly resource-limited communities, should pay more attention to this female population in their fifties during cervical cancer screening and HPV genotyping. Among the HPV genotypes, HR-HPV is the most implicated in the development of precancerous lesions. HPV16 and 18 are the most common genotypes worldwide [7]. In this study, 17 of the 23 women were infected with at least one high-risk oncogenic genotype. HPV35 was the most frequent genotype in this study, followed by HPV18/52/58/68, contrary to the worldwide trend. The distribution of HPV genotypes depends on the population and locality [30]. In the systematic review carried out in Burkina Faso by Zohoncon *et al.*, HPV 18 and 33 were the predominant genotypes, but HPV 35 and 68 were not negligible [20]. Infection with HPV-52, 56, 35, 58 has been reported in studies from several African countries among sexually active women, according to Ouédraogo *et al.* in a robust systematic review in West Africa [30]. HPV 52, which was not covered by available vaccines, was the most prevalent. The most prevalent HPV type was HPV-16 in all cervical cytological abnormalities, followed by HPV-52 and HPV-58 in Shantou, Guangdong province [17].

Vaccination remains the primary means of preventing and eradicating this global scourge. In over 130 countries, HPV vaccination is now included in the national program. In fact, in Burkina Faso, since April 26, 2020, the quadrivalent anti-HPV vaccine Gardasil® 4 has been introduced into the expanded vaccination program for girls aged 9 to 13. It offers protection against HPV genotypes 6, 11, 16 and 18. Our study reveals a low coverage (28.13%) of the Gardasil 4 available in Burkina. These results are similar to those of Kabre *et al.* who reported coverage of 31.04% [14]. Among the three licensed vaccines, the nonavalent Gardasil® 9 clearly showed the broadest coverage on the genotypes identified in women in the general population, with a prevalence of 55.8% according to Ouédraogo *et al.* It is, therefore, necessary for low-income countries to make available the new NanoValent HPV vaccine Gardasil®9, which is currently available in developed countries and which offers protection against 9 genotypes (HPV 6/11/16/18/31/33/45/52/58), in order to combat cervical cancer more effectively.

5. Conclusion

Although this study is relevant because of the consequences of HPV infection on women's health, due to the sample size, the conclusions cannot be generalized for the entire female population of Burkina Faso. It is the first survey on the prevalence of HPV infection and the distribution of HPV genotypes in women with abnormal cytological test results. These data highlight the need for increased surveillance and targeted prevention strategies in this target population of women in

Burkina Faso.

Authors' Contribution

Study design: PZ and TS; Data collection: PZ and ST; Performance and reading of smears: VK and AT; HPV genotyping: PZ, PB, MK, IT, AS, TEY; Statistical analysis: PZ; Writing, review and editing: PZ, TS, JS, AS, KC, ATY, HGO, WFD, AAZ.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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