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Detection of Human Papillomavirus DNA and E6/E7 mRNA from HPV Genotypes 16, 18, 31 and 33 in Histologically Confirmed Cases of Cervical Cancer and Precancerous Lesions in Burkina Faso

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Abstract

Introduction: Cervical cancer, caused by persistent high-risk human papillomavirus (HPV) infection, remains a global public health problem. The cellular transformation and maintenance of the malignant phenotype of these HPVs are attributed to the viral oncoproteins E6 and E7. **Objective:** This study aims to detect the presence of human papillomavirus DNA and E6/E7 oncoprotein mRNA of HPV genotypes 16, 18, 31 and 33 in cases of cervical cancer and precancerous lesions, histologically confirmed in Burkina Faso. **Methods:** This descriptive cross-sectional study focused on cases of cervical cancer and high-grade intraepithelial neoplasia (CIN) and was conducted from June to December 2022. One hundred (100) samples of fixed and paraffin-embedded tissues were collected from the pathological anatomy and cytology laboratories of hospitals in the capital of Burkina Faso. High-risk human papillomavirus (HR-HPV) DNA was detected using multiplex real-time PCR, while the presence of E6 and E7 mRNA in cervical cancer and high-grade CIN samples was determined using real-time Reverse Transcriptase-PCR (RT-PCR) with TaqMan probes. **Results:** The mean age of women diagnosed with cervical cancer and high-grade CIN was 50.81 ± 13.65 years,

ranging from 22 to 82 years. Cervical cancer and high-grade CIN were positive for at least one high-risk human papillomavirus (HR-HPV) in 80% of cases. The most prevalent genotypes observed were HPV16, 18, 31, and 33, collectively accounting for 70.08% of cases. Of the 89 samples that tested positive for HR-HPV genotypes 16, 18, 31, and 33, 88 (98.88%; 95% CI: [94.58 - 99.94]) were also positive for the presence of mRNA encoding the E6 and E7 oncoproteins of HPV16, 18, 31, and 33. **Conclusion:** In the presence of HPV DNA, testing for E6 and E7 oncoprotein mRNA could serve as a promising biomarker and valuable tool for improved assessment of the progression to cervical cancer.

Keywords

HPV, E6/E7, Cervical Cancer, Precancerous Lesions, Burkina Faso

1. Introduction

Human Papillomaviruses (HPV) are non-enveloped, highly resistant DNA viruses that belong to the *Papillomaviridae* family [1]. HPV infection is the most common sexually transmitted infection (STI) worldwide, with more than half of all adults becoming infected during their lifetime [2] [3] [4] [5]. Based on their oncogenic potential in humans, HPVs are classified into two categories: those with high oncogenic risk (including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), which are associated with cancers of the anogenital and oropharyngeal regions, and low-risk HPVs (such as types 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, and 89), responsible for benign lesions like condyloma acuminata and warts [5] [6].

The persistence of an infection with at least one high-risk HPV is the primary cause of most cervical cancers [7]. Cervical cancer poses a significant public health challenge, particularly in developing countries. In 2018, the worldwide incidence of cervical cancer was estimated at 570,000 cases, resulting in 311,000 deaths [8]. Alarmingly, 85% of these cases and 87% of the deaths occurred in low-income countries [9] [10]. Globally, cervical cancer ranked as the fourth most common cancer among women and a leading cause of cancer-related deaths [8].

High-risk human papillomavirus (HR-HPV) viral DNA has been detected in biopsies of cervical cancer and precursor lesions [11]. The cellular transformation and maintenance of the malignant phenotype in these cases are attributed to the viral proteins E6 and E7 [12]. These proteins interfere with various cellular processes, including cell cycle regulation, apoptosis control, DNA repair, mitotic spindle formation, intercellular adhesion, and mitogenic signal transduction pathways. Their disruptive effects contribute to the development and progression of malignancies [13] [14]. The E6 protein binds to the p53 protein, leading to its degradation and causing dysfunction in the

apoptosis process. Meanwhile, the pRB protein plays a crucial role in cell proliferation [15] [16] [17].

Research confirms a strong association between the development of cervical cancer following HR-HPV infection and the expression of E6/E7 oncoproteins [18] [19].

A link between the expression of E6/E7 oncoproteins and the progression of HR-HPV infection to cervical cancer has been described. Additionally, research indicates that these oncoproteins can serve as diagnostic markers to assess the likelihood and risks of progressing to the invasive cancer stage.

In this pioneering study on HPV oncoproteins conducted in Burkina Faso, our primary objective was to detect the presence of HPV DNA and E6/E7 oncoprotein mRNA from HPV genotypes 16, 18, 31, and 33 in cases of cervical cancer and precancerous lesions, which were histologically confirmed.

2. Methods

2.1. Study Framework

The study was conducted in Ouagadougou, the capital of Burkina Faso. Fixed and paraffin-embedded tissues obtained from cervical biopsies, conization procedures, or hysterectomies, all dating back less than a year, were collected from the pathological anatomy and cytology laboratories of Ouagadougou's hospitals, including the largest reference hospital in Burkina Faso, Centre Hospitalier Universitaire Yalgado Ouédraogo. Subsequently, the specimens were transported to the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA). Here, molecular analyses were performed at the "Laboratoire National de Référence pour le HPV" (LNR-HPV).

2.2. Study Type and Population

This was a descriptive cross-sectional study conducted between June and December 2022. It comprised a collection of one hundred (100) tissue samples, which were fixed and embedded in paraffin. These samples were obtained from cases of cervical cancer and high-grade intraepithelial neoplasia.

2.3. Tissue Sample Collection

Specific codes corresponding to confirmed cases of cervical cancer and high-grade cervical intraepithelial neoplasia were identified by utilizing the hospital anatomy and cytology laboratory registers. These codes were then used to locate the corresponding tissue blocks. Sociodemographic information was also documented. Subsequently, the solid paraffin blocks, containing either biopsy, conization, or hysterectomy specimens, were processed by cutting them into five sections, each no thicker than 20 μm, using a microtome.

2.4. Inclusion and Exclusion Criteria

All fixed and paraffin-embedded tissue samples with a histological diagnosis

confirming cervical cancer or high-grade cervical intraepithelial neoplasia (CIN) were included. Samples with a normal histological diagnosis were excluded from this study.

2.5. DNA/RNA Extraction

DNA and RNA extraction was performed using the "R-Biopharm RIDA® Xtract" commercial kit, following the manufacturer's protocols. The process began with the deparaffinization of archived tissue sections using xylene and alcohol. Initially, 1 mL of xylene was added to the tube containing the tissues, followed by vortexing, a 10-minute incubation at 50˚C, and centrifugation at 14,000 rpm for 2 minutes. This deparaffinization step was repeated. Subsequently, 1 mL of absolute ethanol was added, followed by vortexing and centrifugation at 14,000 rpm for 2 minutes. This ethanol wash step was also repeated, and finally, the sample was air-dried for 15 minutes.

The extraction of nucleic acids followed a series of principles and steps, including cell lysis, DNA precipitation (binding of nucleic acids to the column), washing, elution of nucleic acids, and subsequent quantification of the extracts using a Nanodrop. The resulting extracts were stored at - 80˚C for further manipulation.

2.6. Detection of HR-HPV

The detection of HR-HPV was carried out using multiplex real-time PCR, employing the HPV Genotypes 14 Real-TMQuant kit from SACACE Biotechnologies® in Italy. This kit allows for the identification of 14 high-risk HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) by targeting the L1 region of the HR-HPV genome.

For each sample, four PCR tubes were utilized. Each tube contained primers for the target regions of three or four types of HR-HPV and the human beta-globin gene, which served as an internal control to monitor the presence of cellular material in the sample. Specifically, for each sample, we had the following four tubes: PCR-mix-1 (16, 18, 31, IC); PCR-mix-1 (39, 45, 59, IC); PCR-mix-1 (33, 35, 56, 68); PCR-mix-1 (51, 52, 58, 66).

The pre-PCR steps involved the preparation of the Mix solution (PCR-buffer-FRT + Hot Start DNA Polymerase) and the Reaction Mix solution (Mix solution + each PCR-mix-1). For each sample, 15 µL of the Reaction Mix solution was added to the four tubes, followed by the addition of 10 µL of the DNA extract. This resulted in a total reaction volume of 25 µL for each PCR.

These PCR reaction mixtures, contained in sterile 0.2 mL microtubes, were introduced onto the SaCycler-96 Real-Time PCR v.7.3 plate (Sacace Biotechnology, Italy) for amplification. The amplification program consisted of one cycle of 95˚C for 15 minutes, followed by five cycles of 95˚C for 5 sec, 60˚C for 20 sec, and 72˚C for 15 sec, and finally 40 cycles of 95˚C for 5 sec, 60˚C for 30 sec, and 72˚C for 15 sec.

The results were interpreted using Microsoft Excel software and a program

named HPV Genotypes 14 Real-TM.xls (SACACE Biotechnologies®, Italy), following the manufacturer's protocol.

2.7. Detection of HR-HPV Oncoprotein E6 and E7 Transcripts

The detection of E6 and E7 oncoprotein mRNA was conducted in samples that tested positive for HPV DNA. The real-time reverse transcriptase-PCR (RT-PCR) technique, utilizing TaqMan probes, was used to detect the presence of E6/E7 mRNA specifically from HPV genotypes 16, 18, 31, and 33 in cervical cancer and high-grade CIN samples.

2.7.1. Synthesis of cDNA

After mRNA extraction, cDNA synthesis was performed using the M-MLV Reverse Transcriptase Kits from Invitrogen and Random hexamers (Invitrogen), following the manufacturer's protocol. In the initial Mix, comprising 1 µL of dNTP mix (10 mM of dATP, dGTP, dCTP, and dTTP at neutral pH), 0.1 µL of random hexamers, and 5 µL of distilled water, we added 10 µL of RNA. The reaction mixture was incubated at 65˚C for 5 minutes and then rapidly cooled on ice. A second Mix was prepared, consisting of 4 µL of 5× FirstStrand Buffer, 2 µL of 0.1 M dithiothreitol (DTT), and 1 µL of M-MLV reverse transcriptase (RT). The cDNA synthesis proceeded with the following amplification program: 25˚C for 10 minutes, 37˚C for 50 minutes, and 70˚C for 15 minutes.

2.7.2. Amplification by Real-Time PCR

The obtained cDNA was diluted 1/1000 before being utilized for real-time PCR. Real-time PCR for HPV 16/17/31/33 oncoproteins E6/E7 was performed in a total reaction volume of 20 µL, consisting of 10 µL of TaqMan® Universal Master Mix II no UNG, 2X, 0.5 µL of forward and reverse primers, 0.5 µL of probes, 5 µL of cDNA (ranging from 1 to 100 ng), and 4 µL of distilled water. During the manipulation, both a negative control and a positive control were included. The amplification program included an initial denaturation step at 95˚C for 10 minutes, followed by 45 cycles of denaturation at 95˚C for 15 seconds and annealing/extension at 60˚C for 60 seconds. Table 1 provides the sequences of primers and probes used for real-time PCR.

2.8. Data Analysis

Our data were analyzed using Excel, SPSS 20.0, and Epi Info 7.2.2.6 software. We set the confidence interval at 95%, and for comparisons, we employed the Chi-square test. Statistical significance was defined at $P < 0.05$.

2.9. Ethical Considerations

This study received approval from the institutional ethics committee of "Hospital Saint Camille in Ouagadougou", Burkina Faso through its deliberation No. 2022-05-012 of 05/20/2022. Confidentiality and anonymity were strictly respected.

Table 1. Sequences of primers and probes used for real-time PCR.

Primer or probe	Sequence (5' to 3')	Location	Product size (bp)
HPV 16 E6-F	GCACCAAAAGAGAACTGCAATGTT	85 - 108	152
HPV 16 E6-R	AGTCATATACCTCACGTCGCAGTA	197 - 236	
HPV 16 E6-P	GGACCCACAGGAGCGACCCAGAAAGTTA	112 - 139	
HPV 16 E7-F	CAAGTGTGACTCTACGCTTCGG	738 - 759	81
HPV 16 E7-R	GTGGCCCATTAACAGGTCTTCCAA	796 - 818	
HPV 16 E7-P	TGCGTACAAAGCACACACGTAGACATTCGT	763 - 792	
HPV 18 E6-F	CTATAGAGGCCAGTGCCATTCG	503 - 524	79
HPV 18 E6-R	TTATACTTGTGTTTCTCTGCGTCG	558 - 581	
HPV 18 E6-P	CAACCGAGCACGACAGGAACGACTCCA	530 - 556	
HPV 18 E7-F	TAATCATCAACATTTACCAGCCCG	721 - 744	113
HPV 18 E7-R	CGTCTGCTGAGCTTTCTACTACTA	810 - 833	
HPV 18 E7-P	CGAGCCGAACCACAACGTCACACAATGTT	745 - 774	
HPV 31 E6-F	AAGACCGTTGTGTCCAGAAG	428 - 447	106
HPV 31 E6-R	GTCTTCTCCAACATGCTATGC	511 - 534	
HPV 31 E6-P	CGTCCTGTCCACCTTCCTCCTATG	488 - 511	
HPV 31 E7-F	TGTGTTAGATTTGCAACCTGAG	592 - 613	78
HPV 31 E7-R	ACATCCTCCTCATCTGAGCT	669 - 649	
HPV 31 E7-P	CAACTGACCTCCACTGTTATGAGCAAT	615 - 641	
HPV 33 E6-F	TGCACGACTATGTTTCAAGAC	100 - 120	132
HPV 33 E6-R	CTCAGATCGTTGCAAAGGTTT	211 - 231	
HPV 33 E6-P	ATTCCACGCACTGTAGTTCAATGTTGT	179 - 205	
HPV 33 E7-F	TTGTAACCTGTTGTCACACTTG	733 - 754	88
HPV 33 E7-R	AGTAGTTGCTGTATGGTTCGTA	799 - 820	
HPV 33 E7-P	ACTTGCTGTACTGTTGACACATAAACGA	767 - 794	

F: forward; R: reverse; P: TaqMan probe.

3. Results

3.1. Sociodemographic Characteristics of the Study Population

This study focused on a total of 100 archived tissue samples obtained from the cervix. These tissue samples were fixed and embedded in paraffin. Among the samples, the pathological diagnosis revealed 8 cases of CIN2 (Cervical Intraepithelial Neoplasia Grade 2), 8 cases of CIN3 (Cervical Intraepithelial Neoplasia Grade 3), and 84 cases of invasive cervical cancers.

The age of the patients included in this study ranged from 22 to 82 years, with a mean age of 50.81 ± 13.65 years. Most of these patients, accounting for 70% of the sample, were identified as housewives and 41% resided in rural areas. Regarding marital status, 61% of the cases were brides. In terms of educational

background, 80% of the participants had an educational level equal to or lower than primary school. Furthermore, 44% of the cases were classified as large multiparous (having had five or more deliveries), followed by multiparous individuals (having had three or four deliveries) in 30% of cases, with delivery extremes ranging from 2 to 10. Approximately 62% of the patients reported having their first sexual intercourse before the age of 18, with 20% of them experiencing their initial sexual encounter during their pediatric years (between 13 and 15 years of age). The mean age at first sexual intercourse was 16.85 ± 1.69 years, ranging from 13 to 22 years. Surprisingly, 72% of the patients reported never using condoms during sexual intercourse, and they also did not use oral contraceptives. Among the 84 patients diagnosed with invasive cervical cancer, two of them were under 30 years old. Table 2 presents the distribution of the age group according to the histological profiles of cervical cancer.

3.2. Genotyping of HR-HPV and Detection of the Presence of E6 and E7 mRNA

Among the 100 cases of cervical cancer and high-grade CIN (Cervical Intraepithelial Neoplasia), 80 samples tested positive for at least one of the 14 sought HR-HPV (High-Risk Human Papillomavirus) genotypes. This corresponds to a detection rate of 80% when considering viral DNA detection. The cumulative count of detected genotypes reached 127, with the predominant genotypes being HPV16, 18, 31, and 33, collectively representing a cumulative frequency of 70.08% (89 out of 127). The remaining genotypes (35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) constituted 29.92% (38 out of 127) of the total.

The investigation for the presence of E6/E7 mRNA focused on the four most prevalent genotypes, namely HPV16, 18, 31, and 33. However, the cumulative frequency of these seven high-risk genotypes (HPV16, 18, 31, 33, 45, 52, 58) accounted for 80.31% of the genotypes associated with precancerous and cancerous lesions within our study cohort. Therefore, by considering these seven high-risk genotypes covered by the nonavalent vaccine, our findings suggest that the vaccine could potentially prevent 80.31% of precancerous and cancerous lesions, based on the data derived from our study. Figure 1 presents the distribution of the 14 HR-HPV genotypes in the study population.

The positivity rate of E6/E7 oncogene mRNA expression was compared to that of HPV DNA. Among the 89 samples that tested positive for HR-HPV 16, 18, 31, and 33 using the DNA-HPV technique, 88 (98.88%; 95% CI: [94.58 - 99.94]) were also found to be positive for the presence of mRNA for the E6 and E7 oncoproteins of HPV16, 18, 31, and 33. For these four most frequent HR-HPV genotypes, a remarkable 100% concordance was observed between detection via the HPV DNA technique and the detection of the presence of E6/E7 oncogene mRNA in cases of histologically confirmed invasive cervical cancer. Specifically, there were 74 cases positive for both HPV 16, 18, 31, and 33 DNA and HPV 16, 18 E6 and E7 mRNA.

Table 2. Distribution of the age group according to histological profiles of cervical cancer.

Age group (years)	Histological profiles			Total	P-value
	CIN2 n (%)	CIN3 n (%)	Invasive cancer n(%)		
<30	02 (50)	00	02 (50)	04 (100)	0.223
30 - 55	05 (9.1)	07 (11.3)	50 (81)	62 (100)	<0.0001
>55	01 (3.3)	01 (3.3)	32 (94)	34 (100)	<0.0001
Total	8 (100)	8 (100)	84 (100)	100 (100)	

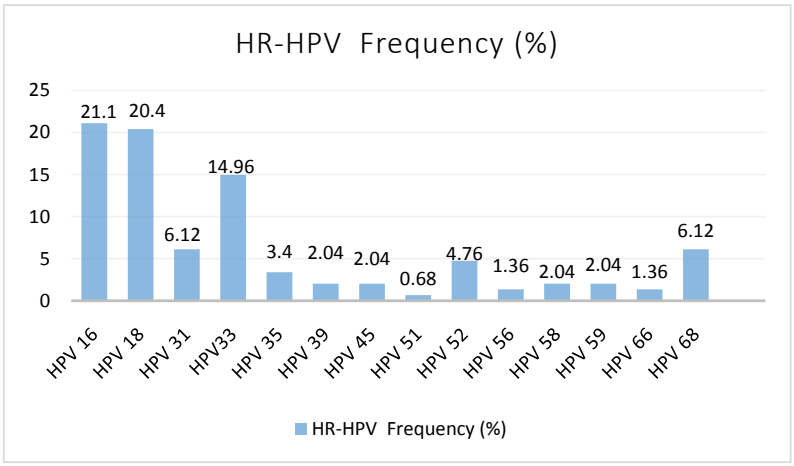

Figure 1. Distribution of the 14 HPV-HR genotypes in the study population.

Furthermore, in the case of CIN2 (Cervical Intraepithelial Neoplasia Grade 2), 87.5% (7 out of 8) exhibited the expression of E6 and E7 mRNA transcripts. In CIN3 cases, a complete 100% expression of E6 and E7 mRNA was observed. When considering high-grade precancerous and cancerous lesions of the cervix, there was no statistically significant difference between the presence of HPV-DNA and the detection of HPV E6 and E7 mRNA for genotypes 16, 18, 31, and 33 ($p = 0.999$). Table 3 shows the frequencies of HPV-DNA and E6/E7 mRNA according to the frequencies of HR-HPV 16, 18, 31, and 33 genotypes and the histological profile of the cervical lesions.

3.3. HR-HPV Genotypes According to the Grade of Cervical Lesions

The most prevalent HR-HPV genotypes in cases of invasive cervical cancer were 16, 18, and 33. Notably, there was an absence of E6 and E7 mRNA expression from HPV 18 in CIN 2 and 3. Table 4 presents the distribution of E6/E7 mRNA from HR-HPV16/18/31/33 according to the grade of cervical lesions.

4. Discussion

Cervical cancer is among the malignancies that can be prevented through understanding the causative agent, namely HPV, and by employing available prevention and screening methods. Nevertheless, it remains evident that cervical

Table 3. Presence of HPV-DNA and E6/E7 mRNA depending on the frequencies of HR-HPV 16, 18, 31 and 33 genotypes and the histological profile of cervical lesions.

	HPV-DNA n (%)	E6/E7 mRNA n (%)	P-value
HR-HPV genotypes			
HPV16	31 (34.83)	31 (35.23)	
HPV18	30 (33.71)	30 (34.09)	0.999
HPV31	09 (10.11)	09 (10.23)	
HPV33	19 (21.35)	18 (20.45)	
Total	89 (100)	88 (100)	
Histological stage			
CIN2	08 (08.99)	07 (07.95)	
CIN3	07 (07.86)	07 (07.95)	0.970
Cancer	74 (83.15)	74 (84.10)	
Total	89 (100)	88 (100)	

Table 4. Distribution of E6/E7 mRNA from HR-HPV16/18/31/33 depending on the histological profile.

E6/E7 mRNA	Grade of cervical lesions			
	CIN2 n (%)	CIN3 n (%)	Cancer n (%)	Total
HPV16	04 (57.14)	04 (57.14)	23 (31.08)	31
HPV18	00 (00)	00 (00)	30 (40.54)	30
HPV31	01 (14.29)	01 (14.29)	07 (9.46)	09
HPV33	02 (28.57)	02 (28.57)	14 (18.92)	18
Total	07 (100)	07 (100)	74 (100)	88

cancer represents a significant global public health challenge, despite the high likelihood of viral clearance in cases of infection. Indeed, persistent HR-HPV infection stands as the primary risk factor for the development of cervical intraepithelial lesions and eventual cervical cancer [20]. While there is evidence of the presence of HPV DNA, the crucial question remains: will the infection be transient or persistent, potentially leading to cervical cancer? Several authors have pointed to a strong association between cervical carcinogenesis and HPV infection, particularly when there is transcription of HR HPV oncogenes E6 and E7, leading to an increase in their mRNA and protein levels. Therefore, the detection of HR HPV E6 and E7 mRNAs holds promise as a valuable biomarker for assessing their persistence and oncogenic activity. This, in turn, could significantly enhance our ability to evaluate the progression to high-grade cervical intraepithelial lesions and may have a substantial impact on the patient monitoring protocol [20] [21]. As per Derbie *et al.*, emerging evidence suggests that HPV E6/E7 mRNA testing may serve as a valuable tool in comparison to cytology and HPV

DNA testing for the early detection of precancerous dysplasia [21]. In addition, Nikolic *et al.* reported that the percentage of expression of HR-HPV oncoproteins E6 and E7 would be proportional to the degree of severity of the cervical lesion. [20].

In our study, we detected 14 HR-HPV DNA in women diagnosed with CIN 2, CIN 3, and invasive cervical cancer. We specifically investigated the most prevalent genotypes, namely HPV 16, 18, 31, and 33, for the expression of E6 and E7 mRNA transcripts. Notably, the average age of women with cervical lesions in our study was 50.81 ± 13.65 years. This finding contrasts with the results reported by Ouédraogo *et al.* in cases of high-grade CIN in Burkina Faso, where the average age was reported as 41.5 ± 9.8 years [22]; Zohoncon *et al.*, (46.32 ± 12.76 years) in cases of invasive cervical cancer in Burkina Faso [23] and Zohoncon *et al.*, (40.05 ± 13.99) in Benin in cases of high-grade CIN and invasive cervical cancer [24]. These differences may be explained by sample sizes, types and grades of cervical lesions, and regions.

In our study, HR-HPV DNA was found in 80% of cases of high-grade CIN and invasive cervical cancer. This result corroborates those of Zohoncon *et al.*, (76.92%) [24] and Sanou-Lamien *et al.*, (76%) [25]. In women with high-grade precancerous lesions, previous authors reported a 70% positivity rate for HPV in 2016 [26]. However, our result is lower than the 48.8% reported in 2016 by Ouédraogo *et al.*, in cases of high-grade precancerous lesions [22].

The most prevalent HR-HPV genotypes observed in our study among cases of high-grade CIN and invasive cervical cancer were HPV 16, 18, 31, and 33. These findings align with results reported by other authors. For instance, Zohoncon *et al.*, conducted a study in Burkina Faso and reported that HPV 18, 31, 16, 39, and 45 were the most commonly found genotypes in cases of invasive cervical cancer, which is consistent with our observations [23].

Nikolic *et al.*, reported a frequency of HPV 16 of 76% in high-grade CIN, followed by HPV 31 (17%) and HPV 33 (9%) [20]. According to these authors, there appears to be a decrease in the frequency of HPV 31 with the progression of precancerous cervical lesions. They suggest that its prevalence tends to be lower in cases of cervical cancer compared to precancerous lesions.

A similar trend is observed for HPV 33, which is also classified fourth in terms of frequency and is responsible for approximately 4.2% of all cases of cervical cancer [20]. Par contre dans notre étude, la prévalence du HPV 33 était nettement élevée. However, in our study, the prevalence of HPV 33 was notably higher. This discrepancy could be attributed to various factors. One possible explanation is the geographic diversity in HPV genotypes [27] [28]. Additionally, the phenomenon of viral clearance may play a role in these differences. In their study, Nikolic *et al.* reported a low frequency of HPV genotypes 52, 56, 45, 18, 59, 58, 39, 35, and HPV 18 [20]. However, HPV 18 is considered to be responsible for approximately 15% of invasive cervical cancers.

The frequency of HPV 18 has been reported to be 10 times lower than that of

HPV 16 [20]. However, this was not the case in our study, as HPV 18 was the most prevalent (40.54%) among the four predominant genotypes in cases of cervical lesions. Clifford *et al.* had reported that only HPV 16, HPV 18, and HPV 45 represented a larger proportion of HPV infections in cases of invasive cervical cancer compared to cases with normal cytology [29]. Nevertheless, in our study, HPV 18 was not detected in cases of CIN. This aligns with the findings of Kusakabe *et al.*, who reported that HPV 18 is challenging to detect in precancerous lesions [16].

To date, not all HR-HPV genotypes are covered by the available HPV vaccines. HPV vaccination has been introduced in more than half of the World Health Organization (WHO) member countries as of 2020 [20]. This became a reality in Burkina Faso on April 26, 2022, with the introduction of the HPV vaccine for girls aged 9 to 14 years [30]. According to Nikolic *et al.*, the results of their study indicate that 80% of precancerous lesions could be prevented using the nonavalent vaccine [20]. These findings align with the outcomes of our research, as our data suggests that the nonavalent vaccine could potentially prevent 80.31% of cervical lesions.

Malla and Kamal, in 2021 reported that cervical cancer is the fourth most common cancer among women aged 15 to 44 worldwide [17]. Experimental and epidemiological studies have identified that HPV types 16 and 18 are responsible for 70% of precancerous cervical lesions and cervical cancer worldwide due to their ability to induce genetic and epigenetic alterations in the host genome [17].

Some studies have reported the presence of E6 and E7 mRNA in women with normal cervical cytology [20] [31]. Valença *et al.*, in 2016 reported that the E6/E7 test was positive in 69 samples (57.5%) with negative cytological results [26]. HPV 16 E6/E7 mRNA was detected in 61.2% of cases, followed by HPV 33 (26.5%), HPV 31 (17.3%), and HPV 18 (10%) [26].

The positivity rate of mRNA for oncoproteins E6 and E7 of HPV16, 18, 31, and 33 was 98.88% in our study. Similarly, other authors have reported high positivity rates of E6/E7 mRNA in cases of CIN and cervical cancer. For instance, Valença *et al.*, reported in their study that out of 111 women who underwent biopsy and an E6/E7 test, the positivity rate of the E6/E7 test was 84.7% in women with cervical intraepithelial neoplasia (CIN) grade 2 or higher lesions [26]. In addition, Yu and Wang 2022 reported that the presence of HPV DNA and HPV E6/E7 mRNA were significantly higher in cases of CIN and cervical cancer than in cases of inflammation of the cervix [32]. Yu and Wang also reported that the specificity of HPV E6/E7 mRNA was lower than that of HPV DNA in detecting low-grade intraepithelial neoplasia, but it performed better in detecting high-grade intraepithelial neoplasia and cervical cancer [32]. Furthermore, Cao *et al.* reported that the total E6/E7 mRNA positivity rate was 67.27% and there was no significant difference between CIN1 and CIN2/P16 negative [33]. All of the above findings support the notion that the mRNA test for HR-HPV oncoproteins E6 and E7 could serve as a potential biomarker to facili-

tate early diagnosis and, consequently, the early management of high-grade intraepithelial cervical lesions and cervical cancer.

5. Conclusion

The present study has successfully demonstrated the presence of HR-HPV DNA and the expression of E6 and E7 oncoprotein mRNA from HR-HPV 16/18/31/33 in cases of high-grade cervical intraepithelial neoplasia and invasive cervical cancer. The detection of HR-HPV oncoprotein E6 and E7 mRNA represents a promising biomarker that could significantly enhance the assessment of progression toward high-grade cervical intraepithelial lesions and cervical cancer. The incorporation of routine tests for HR-HPV oncoprotein E6 and E7 mRNA could provide a valuable tool for early diagnosis and timely management of cervical cancer. This advancement holds great potential for clinicians, offering improved diagnostic accuracy and ultimately benefiting patients in the ongoing battle against this devastating disease.

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Conflicts of Interest

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

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