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Circulating High-risk HPV Genotypes in the South of Benin and Disparity with General Immunization Target

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Abstract **Background** High-Risk Oncogenic Human Papillomaviruses (HR-HPV) are accountable for 7.7% of cancers in developing countries, mainly cervical lesions. In Benin, HR-HPV infection in women triggered nearly 781 new cases of cervical cancer each year leading to 616 (79%) deaths. Current vaccines may not cover all HR-HPV genotypes encountered in West Africa including Benin. The objective of our study was to determine HR-HPV genotypes in the South of Benin to launch regional HPV mapping associated to cervical lesions. For this purpose, HR-HPV genotypes from 2017 was compared to HPV genotypes from 2007 in the south of Benin to evaluate HR-HPV trend over a decade along with associated cervical lesions. **Methods:** regardless of technical methods**,** retrospective comparative analysis was done on HR-HPV genotypes of cervical uterine swab (CUS) samples in 2017 (n= 234) and 2007 (n=385). In 2017 real-time multiplexed PCR was used while in 2007-traditional nested polymerase chain reaction (PCR) was used. In both cases screening of cervical precancerous and cancerous lesions (dysplasia) was performed by colposcopy subsequently to vaginal application of acetic acid (VIA) and Lugol's iodine solution (VILI). Statistical analysis was done with Pearson chi2 (χ^2) test proportions and Student test. The difference was considered statistically significant for $p < 0.05$. **Results:** The prevalence of HPV infected women in 2017 was 34% with 30 co-infections. Overall HR-HPV count was 125 with high frequency for HPV52 (16%), HPV58 (10%), HPV51 (9%), HPV66 (8%), HPV68 (8%), HPV35 (8%) and HPV45 (8%). The least frequent genotypes were HPV18 (6%), HPV16 (1.6%) and HPV33 (1.6%). Positive VIA and VILI were observed respectively in 5.55% (13/234) and 6.83% (16/234) women. In 2007 the prevalence of HR-HPV was 22.07% with 18 co-infections. Common HR-HPV genotypes found were HPV 35 (14.28%), HPV31 (13.33%), HPV66 (13.33%), HPV68 (13.33%), HPV58 (10.47%), HPV52 (8.57%), HPV51 (7.61%), HPV18 (6.66%), HPV45 (5.71%), HPV16 (3.80%) and HPV33 (3.80%). No correlation was observed between HR-HPV and cervical lesions. **Conclusion:** HR-HPV infection keep rising over a decade in the south of Benin with noticeable disparity with cervical lesions. Regional HR-HPV trend should be investigated prior to large scale vaccination for cervical cancer prevention in Africa.

Keywords: *Oncogenic HPV, genotypes, vaccine targets, cervical lesions, disparity, south of Benin*

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1. Background

High Risk Human Papilloma Viruses (HR-HPV) are small viruses infecting epithelial cells leading in most cases to cervical cancer when they infected the genital tract epithelium [1]. HPV infections are classified among the three most common sexually transmitted infections along with genital herpes, Chlamydia trachomatis infections and affect 75 to 80% of sexually active people [2]. Infection with Oncogenic HR-HPV is one of the leading causes of cervical cancer in developped countries [2].

The most common HR-HPVs studied were HPV16, 18, 31, 33, 35, 45, 52, 58 and 59. They are presumed to be responsible for 90% of cervical cancers with 70% of them associated to HPV16 and 18 [3]. In 2010, the prevalence of HPV infection was estimated to be 24% in Sub-Saharan

African countries and 14% in Asia [4]. In Benin, cervical cancer ranked second after breast cancer with nearly 781 new cases occuring annually associated to 79% of mortality [5]. According to the World Health Organization (WHO), by 2030 cervical cancer will kill annually more than 443,000 women worldwide with nearly 90% of them in sub-Saharan Africa [3].

The worsening epidemiological state of cervical cancer in Sub-Saharan Africa was largely due to the absence of coherent and integrative national prevention and medical care programs. To prevent HPV infection, three vaccines are currently registered by the European Medicines Agency (EMA): bivalent vaccine (Cervarix®) (bHPV) , quadrivalent vaccine (Gardasil®) (qHPV), and nonavalent vaccine (Gardasil® 9) respectively directed against "HPV16 and 18" ;" HPV 6/11, 16 and 18" ; "HPV 6/11, 16, 18, 31, 33, 45, 52 and 58".

Girls under 9 years are the primary receiver of current vaccines which may protect them before their first sexual intercourse [6]. These vaccines showed almost 70% of coverage for HPV related cervical pre-cancerours and cancerous lesions [6]. In 2016, a pilot population-based HPV vaccination program was conducted in the southern Benin among girls between 9-13 years. These girls should be monitored annually regarding their HPV statut and cervical dysplasia for at least ten years to recognize the efficacy of the immunization in the context of the HPV genotypes encountered in Benin.

The prerequisite for vaccination is an integrative epidemiology of HPVgenotypes circulating in a given region. The objective of our present study was to determine HR-HPV genotypes frequently encountered in the south of Benin (Cotonou and surrounding areas) to establish accurate regional HPV mapping and their association with cervical lesions by taking into consideration the trend of HPV genotypes within a decade 2007 to 2017 (regardless of screening technics), all of which will contribute to chaperon population-based vaccination.

2. Methods

2.1. Reagents

In 2007 study, Phenol/chloroform and ethanol used for DNA extraction were from Sigma Aldrich (France). HPV primer mixtures used contained 3 consensus oligonucleotide sequences (Cust: GELLY G/ Name: GP-E6-3F ; GP-E6-3B ; GP-E6-6B) to detect viral genome and included 14 pairs of forward and reverse primers for specific HPV genotype. These primers used for HPV analysis were purchased from Eurogentec (England). Biometra T3 Thermal Cycler was used for Nested PCR reaction. For electrophoresis, agarose powder, Ethylenediaminetetraacetic acid (EDTA), DNA ladder (100bp) and ethidium bromide used were from Sigma-Aldrich (France).

In the 2017 study, SACACE biotechnologies® DNA-Sorb-A kit was used for DNA extraction, HPV Genotypes 14 Real-TM Quant," code V67-100FRT and the Sacycler-96 Real-Time PCR (SACACE Biotechnologies, Como, Italy) were used to detect 14 high-risk HPV genotypes from our samples.

In both studies in 2007 and 2017, visual inspection with acetic acid and Lugol iodine (Sigma-Aldrich Europe) were used for cervical precancerous lesions screening.

2.2. Population

This study was mainly carried out in two (02) hospitals located in the city of Cotonou, National University hospital (CNHU-HKM) and Zonal University hospital of Mènontin. In 2017 the population was composed of 234 sexually active Beninese women over the age of 18 who agreed to participate in the study during gynecological checkup or voluntary cervical lesions screening. The retrospective data used are derived from results obtained among 385 women with vaginal inflammation who agreed to participate in histological and nested PCR screening study carried out in 2007.

In both cases signed informed consents were obtained prior to CUS sample collection. Clinical data and other variables were collected using an individual collection sheet signed by each participant. Our exclusion criteria were women and girls who were virgins, pregnant, undergoing total hysterectomy or had their period. Our inclusion criteria were girls or women over 18 years, apparently healthy and had never been screened for HPV.

2.3. Type of Study, Ethics Approval and Consent to Participate

This study is a descriptive cross-sectional study with retrospective data analysis. Institutional ethical approvals (CER-ISBA and CER-Parakou) were obtained prior to the study [19,20]. Signed informed consents regarding sample analysis and publication of data were obtained from all participant prior to sample collection.

2.4. Cervical Uterine Smear Collection

Speculum and cytobrush were used to collect CUS as previously reported [7-18]. Briefly a disposable sterile speculum was introduced in the vagina along with a disposable cytobrush to reach the cervical-uterine junction where epithelial cells were collected by rotating the cytobrush clockwise twice. The brush was placed in a dry sterile ice-cold collection tube and kept at -20°C until DNA extraction.

2.5. DNA Extraction

DNA from 2007 samples was extracted by the phenol/chloroform method. Cells lysis buffer along with inhibitors of RNA and proteins (proteinase K at 20 mg/ml) have been used to obtain pure DNA. Phenol was added to the cell lysate volume per volume (V/V) and centrifuged at 10,000 rpm/4 °C for 10 min to collect the supernatant in another Eppendorf tube before adding chloroform V/V followed by another centrifugation at the same speed. The supernatant from this centrifugation was collected in a new Eppendorf tube and DNA was precipitated with ice cold ethanol (96 %) at -20 °C for 4 hours. The tube was centrifuged again at 12,000 rpm/4°C for 10 min and the DNA pellet was washed with 70% ice cold ethanol.

The DNA pellet was air dried at 55°C and eluted with tris-EDTA (TE) buffer to obtain soluble DNA.

After extraction, the purity of the samples was evaluated by measuring the optical density (OD) at 260 nm and 280 nm to determine the OD ratio 260/280. The DNA extract was considered pure if the OD ratio was in the range 1.5-1.9. Soluble DNA ready for analysis was stored at -20°C until needed for PCR [20].

For 2017 samples, HPV viral DNA extraction was performed by using the SACACE biotechnologies® DNA-Sorb-A kit according to the protocol provided by the manufacturer. Briefly 100 µl of CUS in suspension was lysed with 300 µl of lyse solution and incubated for 5 minutes at 65°C. After centrifugation, 20 µl of "*sorbent*" were added and incubated at room temperature. After membranes and proteins lysis, the DNA extract was washed and eluted to get DNA ready for PCR according to the manufacturer protocol.

2.5.1. Real-time PCR Reaction

The 2017 study is the continuation of a sub-regional study, already conducted in Parakou (Benin) and Burkina Faso, where genotyping was performed by real-time PCR. Hence, HR-HPV genotypes were determined by real-time PCR using "HPV Genotypes 14 Real-TM Quant," code V67-100FRT and the Sacycler-96 Real-Time PCR kit. In brief, the PCR performed was a multiplex type with four tubes for each sample. Each tube contained fluorescent primers targeting E6 and E7 regions of three to four variants of HPV genotypes; the β-globin gene was used as internal control. The kit used in this study can detect HPV 16, 39, 33, 58, 31, 45, 35, 52; 18, 59, 68, 66; 51 and 56 as described by previous studies [13,17,22].

We carried out series of 24 reactions as follows: 60 µl of "Hot fast DNA Polymerase" was added to 1.1 ml of "PCR-Buffer-FRT" and mixed by vortex; then a volume of 120 µl of this mixture was aliquoted into four PCR tubes. Each tube contained set of HR-HPV primers for HPV16, 18, 31 in tube 1; primers for HPV39, 45, 59 in tube 2; primers for HPV33, 35, 56, 68 in tube 3 and primers for HPV51,52,58, 66 in tube 4 along with 240 µl of PCR master mix. The final mixture was aliquoted into 96 PCR microtubes (15 µl/tube) and then 10 µl of DNA was added to each PCR tube. The total volume of solution for the PCR reaction was 25 µl. Four positive control loads were prepared in parallel by adding 10 µl of "Positive Controls K1/K2" in four PCR microtubes corresponding to the positive controls. Similarly, four negative controls were included by adding 10 µl of "DNA-Eluent" in four PCR microtubes corresponding to the negative controls. To amplify viral DNA and genotype HPV, DNA were mixed with PCR master mix in PCR microtubes and incubated in a thermocycler according to the following program:

Step 1: 95°C for 15 minutes for enzyme activation x 1 cycle; 95°C for 5 seconds to allow DNA denaturation; 60°C for 20 seconds to allow primers hybridization to DNA (x 5 cycles); 72°C for 15 seconds to start viral DNA elongation; 95°C for 05 seconds to allow DNA denaturation; 60°C for 30 seconds to allow primers hybridization to DNA (x 40 cycles); 72°C for 15 seconds to allow final DNA elongation.

2.5.2. Multiplex Nested PCR Reaction

ß-actin gene was amplified to assess 2007's samples quality before setting up the PCR with HPV multiplex primers for genotyping. To assess the quality of the assay, each PCR reaction has been performed by using positive and negative controls. A first consensus PCR was performed to confirm or not the presence of HPV DNA. Then the product of the first amplification was used again for a second amplification with the set of multiplex primers to determine the genotype of HPV found. The specific genotype screening was divided in 4 groups according to the set of primers. The first set of primers targeted HPV16, 18, 31, 59 and 45. The second set of primers targeted HPV 33, 6/11, 58, 52 and 56. The third set of primers targeted HPV 35, 42, 43 and 44. The set fourth set of primers targeted HPV68, 39, 51, 66. The PCR products were run through 1.5 % agarose gel to separate amplicon according to their size. Amplified bands incubated with ethidium bromide and stained HPV DNA was visualised under UV light. The HPV genotype was determined according to their molecular weight compared to DNA ladder scale [20].

2.6. Visual Colposcopy

Women uterine cervix was examined following successive sprays with 5% diluted acetic acid followed by Lugol's iodine solution to reveal cervical lesions. Acetic acid reacts with cancer cell surface glycoproteins and gives white color (visual inspection with acetic acid, VIAA+) and yellow when stained with Lugol's iodine solution (visual inspection with Lugol's iodine solution, VILI+) as previously reported [19,20].

2.7. Statistical Analysis

The data were processed and analyzed using Epi Info 7.1, XLSTAT and STATA softwares. We characterized the raw link between infection and different explanatory variables using a Pearson chi2 (χ^2) test to compare proportions and a Student test for means. The difference was considered statistically significant for $p < 0.05$.

3. Results

In this present study (2017) we have assessed social and sexual characteristic in Table 1 and 14 HR-HPV genotypes in 234 CUS as well as risk factors for HR-HPV infection. Data are compared to retrospective data obtained in 2007. The results are displayed as follows.

3.1. Influence of Social and Sexual Behaviors on HR-HPV Infections

Social and sexual behaviors of the women in our study are summarized in the Table 1. The age of women in the general population ranged from 18 to 59 years with an average of 32.80 years (SD ± 8.5). We found out that few women under the age of 24 had participated in the study, while women aged from 25 to 34 years were predominant. 48% of them claimed to have never had a previous gynecological consultation (data not shown). Women who

participated in this study were employees, businesswomen, workers from informal sectors, housewives and students. Among the participants, 80.34% had attended middle schools and higher education while 18.26% of them had no education or had primary school education level. The age at the first sexual intercourse ranged from 10 to 28 years, with an average of 18.32 years (SD ± 2.5).

Table 1. Characteristics of the population studied

Characteristics	Number (n)	Percentage (%)
Age		
Group A < 24 years	32	13.68
Group B < 25 - 34 < years	108	46.15
Group C > 35 years	94	40.17
Education Level		
No education level	20	8.55
Primary	22	9.40
Secondary	91	38,9
University	96	41.02
ND	5	2.13
Matrimonial status		
Married	171	73.1
Single	49	20.94
Divorced	3	1.28
Widow	4	1.7
Concubine	3	1.28
ND	4	1.7
Profession		
Informal sector	106	45.3
Jobless	1	0.43
Housewives	10	4.27
Students	18	7.7
Employee	93	39.74
ND	6	2.56
Sexual partners		
1	224	97.74
2	5	2.13
ND	5	2.13
Age at the first sexual intercourse		
<17	76	32.5
18-24	136	58.11
>25	9	3.84
ND	13	5.55
Number of Pregnancies		
0	48	20.51
1-2	97	41.45
3-4	70	29.93
>5	11	4.7
ND	8	3.41
Number of Births		
0	24	10.26
1-2	87	37.2
3-4	81	34.61
>5	34	14.52
ND	8	3.41
VIP		
0	152	64.95
>1	82	35.05

3.2. HR-HPV Genotypes Determination by Real-Time PCR Results

The prevalence of HR-HPV infected women in 2017 was 80/234 (34%) with 30 co-infections. The HPV count was 125 of which the most frequent genotypes were HPV52 with 20/125 (16%); HPV58 13/125 (10%); HPV51 11/125 (9%); HPV66 10/125 (8%); HPV68 10/125 (8%); HPV35 10/125 (8%); HPV45 10/125 (8%); HPV18 8/125 (6.4%); HPV16 (1.6%) and HPV33 (1.6%) as shown in Figure 1. The least frequent genotypes were HPV 18 (6.4%), HPV 16 (1.6%) and HPV 33 (1.6%) as shown in Figure 1. Nevertheless, co-infection with HPV 16 and 18 was found out once. No correlation was observed between HPV infection and cervical lesion ($p>0.05$).

3.3. Risks Factors for HR-HPV Infection

To determine risk factors associated to HPV infection in our population, we investigated the relationship between infection and women's social and sexual behaviors. Thus, the risk factors investigated with HPV positivity are listed in Table 2.

Table 2. Risks factors associated with HPV genotyping

	HPV+ n=80	HPV- n=154	Total n=234	*P*
Age				***0.002**
Group A < 24 years	17	15	32	
Group B < 25 – 34 < years	44	64	108	
Group C > 35 years	19	72	91	
ND	0	3	3	
Age at first sexual intercourse				***0.025**
˂ 18	33	43	76	
≥18	43	102	145	
ND	4	9	13	
Parity				***0.021**
Nulliparous	13	11	24	
Primiparous	19	20	39	
Multiparous	46	117	163	
ND	2	6	8	
Colposcopy VIA/VILI				
VIA				
Positive	3	10		0.387
Negative	77	144		
VILI				
Positive	2	14		0.058
Negative	78	140		

VIA: visual inspection with acetic acid; VILI: visual inspection with Lugol's iodine solution. * $P < 0.05$ and is significant.

3.3.1. HR-HPV Distribution According to the Age at the Study

To facilitate graphical representation, our population of 234 women was subdivided in: group A for women younger than 24 years which represented 13.68% of our population (32/234); group B for women from 25 to 34 years which represented 46.15% of our population (108/234); while group C was for women older than 35 years which represented 40.17% of our population (94/234). Among group A, 47% were HR-HPV negative and 53% were HR-HPV positive. Among group B, 59% were HR-HPV negative and 41% were HR-HPV positive. Among group C, 80% were HR-HPV negative and 20% were HR-HPV positive (Figure 2).

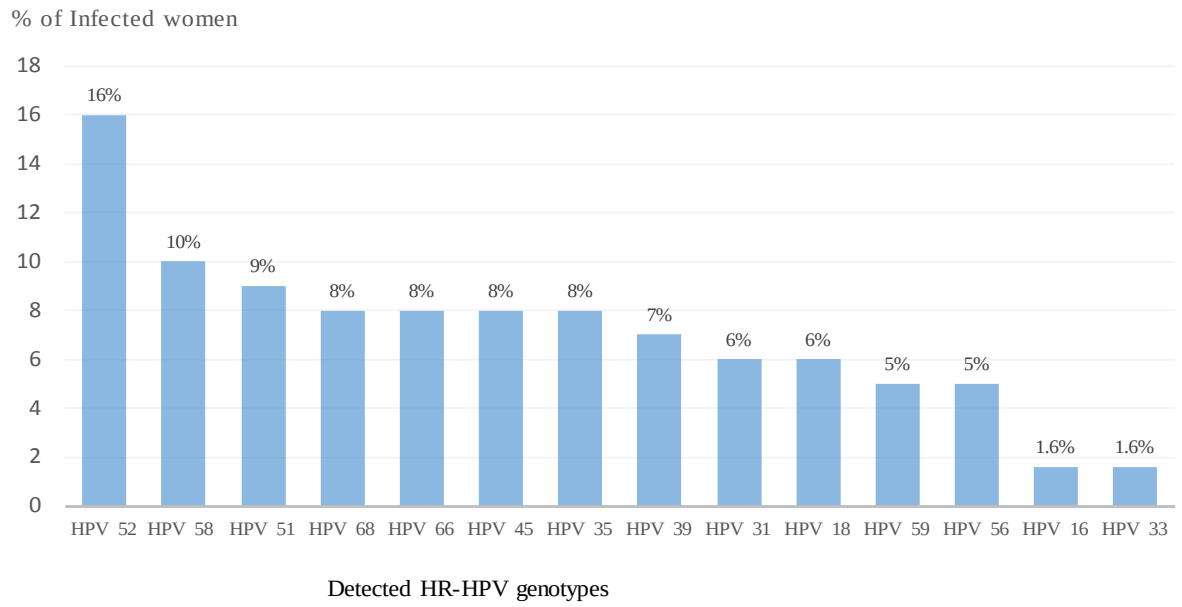

Figure 1: Percentage of circulating genotype in the south of Benin. HR-HPV genotypes detected in the south of Benin. Prevalence of fourteen (14) HR-HPV investigated among the women population studied in the south of Benin in Year 2017 were HPV52 20/125 (16%); HPV58 13/125 (10%); HPV51 11/125 (9%); HPV66 10/125 (8%); HPV68 10/125 (8%); HPV35 10/125 (8%); HPV45 10/125 (8%); HPV18 8/125 (6.4%); HPV16 (1.6%) and HPV33 (1.6%).

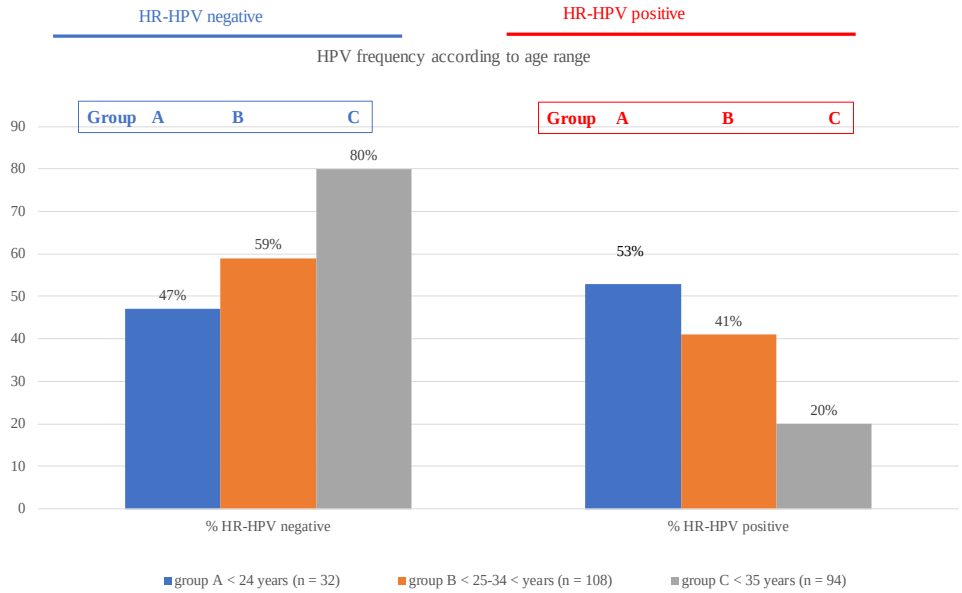

Figure 2. HR-HPV frequency according to age range. In group A we observed 53% with HR-HPV infection; in group B 41% had HR-HPV infection while in group C 20% were positive for HR-HPV. The frequency of HPV was higher in age group A than in group B and group C.

3.3.2. HR-HPV Distribution According to the Age at First the Sexual Intercourse

Following analysis of social and sexual behaviors, we determined that 41.25% of women who had their first sexual intercourse before 18 years were infected with at least one HPV genotype, while 53,75% of those who had their first sexual intercourse at 18 years or older were less infected. Age at first sexual intercourse is inversely correlate to HR-HPV infection (*P = 0.025*).

3.3.3. HR-HPV Distribution According to the Marital Status

Most participants of this study were married or living as couple with men (n= 178). Single women participated less to the study (n = 56). Among the women leaving in couple, 72% were not infected with HR-HPV while 28% of them were infected with at least one type of HR-HPV. Among solitary women, 45% of them were not infected by HR-HPV while 55% were infected with at least one type of HR-HPV. Infection with HR-HPV was significantly higher in solitary women than in women living in couple with men (*P = 0.020*).

3.4. Default of Association between HR-HPV and Pre-cancerous Lesions

Precancerous and cancerous lesions were screened with visual inspection following acetic acid (VIAA) and Lugol

iodine (VILI) staining, the prevalence of cervical lesions was respectively 5.55% (13/234) and 6.83% (16/234). Among the 13 cases with positive staining for VIAA, 3 of them had HR-HPV (3/13); while among the 16 cases stained positive with VILI only 2 of them had HR-HPV (2/16). Positive staining with VIA and VILI indicating the presence of precancerous lesions, was not significantly associated with HR-HPV infection as indicated in Table 2; $P = 0.387$ and $P = 0.587$ respectively.

3.5. Retrospective Data

We compared our HR-HPV genotypes data obtained in 2017 on CUS of seemingly healthy women in the south of Benin and analyzed by Lab Biogen (Burkina Faso), to the data of HR-HPV genotypes analyzed by the laboratory of Biochemistry and Molecular Biology in 2007 on CUS of south of Benin women with vaginal or cervical inflammation (n = 385) in Cotonou, Benin [23]. Histological analysis and Nested PCR were performed on these Inflammatory CUS (ICUS) belonging to the age group of 20-40 years (33%), 41-50 years (40.9%) while barely 1% were under 20 years. Among 385 ICUS collected 131 displayed metaplastic lesions. The prevalence of HR-HPV infection was 22.07% (85/385). A total of 105 HR-HPV counts were found among the 85 infected women, including 18 co-infections. The most commonly found genotypes were HPV 35 (14.28%), HPV31 (13.33%), HPV66 (13.33%), HPV68 (13.33%), HPV58 (10.47%), followed by HPV52 (8.57%), HPV51 (7.61%). The least found genotypes were HPV18 (6.66%), HPV45 (5.71%), HPV16 (3.80%), HPV33 (3.80%). HPV39, 56 and 59 (0.9%).

3.6. Trend of HPV Genotype Evolution within a Decade in the South of Benin

In year 2007 the predominant genotypes were HPV 35 (14.28%), HPV31 (13.33%), HPV66 (13.33%), HPV68 (13.33%), HPV58 (10.47%), HPV52 (8.57%) and HPV51 (7.61%) followed by HPV18 (6.66%), HPV45 (5.71%), HPV16 (3.80%), HPV33 (3.80%).

In year 2017 the predominant genotypes were HPV52 with 20/125 (16%); HPV58 13/125 (10%); HPV51 11/125 (9%); HPV66 10/125 (8%); HPV68 10/125 (8%); HPV35 10/125 (8%); HPV45 10/125 (8%) followed by HPV18 8/125 (6.4%); HPV16 (1.6%) and HPV33 (1.6%).

There was a 1.78-fold decrease in the prevalence of HPV35 and a 1.86-fold increase in the prevalence of HPV52 within a decade. The prevalence of HPV58 remains unchanged in a decade. The prevalence of HPV18 was low in the south of Benin population in 2007 (6.66%) and in 2017 (6.4%). The prevalence of HPV16 although very low in 2007 (3.80%) was lower in 2017 (1.6%); similarly, for HPV33 respectively 3.80% in 2007 down to 1.6% in 2017.

4. Discussion

Cervical cancer is the number one killer among women dying subsequently to cancerous diseases. The odd is that cervical dysplasia in Africa is not always associated to HPV16 and HPV18 infections as it was demonstrated in American and European countries.

The present study shows for the first time the general characteristics of the population studied, then the social and sexual factors linked to HR-HPV infection. Few studies were carried out on HR-HPV in the south of Benin [19,20,23]. Comparative analysis of HPV data we obtained in 2017 from asymptomatic women and data from women with vaginal inflammation in 2007 in the south of Benin showed that in both cases there is no correlation between HPV infection and cervical lesions [23].

The trend of HPV within a decade did not show an increase in the prevalence of HPV18 or HPV16. The average age of women in the study was 32.8 years, which was significantly associated to HPV infection. It is in line with the mean age of 32 years found in a population of 339 women who came for visual screening in the South Abobo General Hospital of Ivory Coast [7]. Women between 25-35 years participated strongly to our study, while women below 20 years were underrepresented (1.71%). This discordance could be explained by the fact that cancer is perceived as a disease affecting elderly and the lack of interest of young girls in our awareness campaigns for HR-HPV and cervical cancer pre-screening. Ideally, women in the age group of 30-50 years were targeted for cervical cancer screening [3]. Our study showed that among women infected with HR-HPV in the south of Benin according to the age group, HR-HPV infection prevalence was higher in women younger than 24 years (53%) than in women between 25-34 years (41%) and women older than 35 years (20%).

We have also found out that most women infected with at least one HR-HPV (77.5%) had their first sexual intercourse before the age of 20 and did not use condoms. Few women of group A participated to the prescreening study because young women were not interested by cervical cancer and presumed that they could not get cancer because they were young. Nevertheless, the frequency of HPV was higher in age group A than in group B and group C (Figure 2). The frequency of HR-HPV infection reported lower in group C could be due to less reproductive sexual activities for their age, which might protect them from sexual transmissible diseases including HPV *(P=0.02)*.

In non-infected women group, the average age at the first sexual intercourse was 18.32 years and is comparable to the average age found out in studies conducted in Burkina Faso and Mexico suggesting that early sexual intercourse may promote HR-HPV infection [8,9]. Overall, mean age at the first sexual intercourse was significantly associated with HR-HPV infection in the south of Benin. The frequency of HR-HPV infection was higher in solitary women group (55%) than in married women or women living in couple with men (28%). Married women or women living in couple with men were less exposed to random sexual activities which may protect them from HR-HPV infection.

The overall prevalence of HR-HPV infection in the south of Benin in 2017 (34.18%) was slightly higher than the prevalence in 2010 (33.2%) reported earlier [24]. It is in line with the prevalence found out recently in Burkina Faso, Cameroon and Madagascar [11-13]. It is higher than

6.19% found in Ivory Coast [7]. The prevalence of HR-HPV in most African countries was lower than the 42.5% reported in United States [14]. The prevalence of cervical lesions in our population was 6.83% with colposcopy examination (VIA/VILI).

Studies conducted in Ivory Coast and in Benin had reported also a discrepancy between HR-HPV infection and cervical lesions [17,18]. Our present study reports the same discrepancy. There was no significant relationship between HR-HPV and VIA/IVL staining. Epithelial cells could have latent HPV without cell transformation into cancer cells thus this could explain the negative VIA or VILI in most HR-HPV positive women. One case of CIN1 and negative HPV was found; leading to the awareness that HR-HPV was not necessarily the factor that trigger cervical lesions in African women [19,20,23]. Previous studies demonstrated that, while cervical lesions were often attributed to oncogenic HR-HPV infection, there were women who had cervical lesions but do not have HR-HPV or HIV infection [18,19,20]. To better assess the role of HR-HPV in cervical carcinogenesis, various (new) biomarkers should be considered for cervical cancer pre-screening and prevention in African countries particularly [18-22].

Indeed, studies showed that nuclear envelop protein lamin A/C deficiency in epithelial cells was a biological process involved in cervical precancerous and cancerous lesions [19,20]. As matter of facts, HR-HPV protein E7 phosphorylates lamin A/C and initiates the disintegration of lamin A filaments essential for the maintenance of nuclear morphology and genome integrity [18,19,20]. Deficiency of lamin A/C promoted abnormal cell divisions, polyploidy/aneuploidy, chromosomal instabilities all of which were hallmarks for cancers [18,19,20]. The suppression of lamin A/C proteins was also implicated in many other cancers [21]. The results of our study carried out 10 years ago showed a lower prevalence of HR-HPV (22.07%) with a predominance of HR-HPV 30S followed by 50S while our current study shows an increase of at least 10% in HPV infection and a predominance of HR-HPV 50S. Other studies conducted in Benin showed the predominance of HR-HPV 50S [18,19,20,24]. In developed countries, HR-HPV immunization programs was introduced since 2006, while developing countries promoted HR-immunization few years back [24].

In Benin, a pilot program was only launched in 2016 with the bivalent vaccine. Many other studies showed that HPV genotypes targeted by bivalent and tetravalent vaccines are different from HR-HPV genotypes found in high proportions in West Africa [13,18,22,23,24]. Although 37.5% of co-infection was observed, most infected women had HR-HPV mono-infection. Among 234 women screened in this study, only 16 of them had precancerous lesion breaking down to 5/16 with HR-HPV (3 HPV58, 1 HPV51 and 1 HPV45). Overall, 2% of women investigated had HPV associated cervical dysplasia while 3% had cervical dysplasia independently of HPV infection as reported earlier in a different study [19,20]. From a public health perspective, the low prevalence of HPV16 and HPV18 in the south of Benin could undermine the predictable efficacy of HR-HPV vaccines to prevent cervical cancer in our region and possibly in other countries of the sub-Saharan Africa.

Surveys conducted in Cotonou have reported non-significant association between cervical lesions and HPV infections in asymptomatic women [18,23]. The same observations were made in our present study and directed us toward other determinants for cervical cancer including epigenetic modifications, non-effective vaginal hygiene and unhealthy diets. All of which could be crucial to unveil further mechanisms and environmental factors involved in the progression of genital HR-HPV infections and cell transformation leading to cancer. Further investigation is needed to understand why some women do not develop precancerous and cancerous lesions despite the presence of multiple HR-HPV [18,19,20,23]. In summary, even though HPV16 and 18 are common in developed countries where they were reported to be responsible for most cervical cancers, this association was not yet demonstrated in west Africa and inflamatory vaginal smears does not always associate with cervical lesions [18,19,20,23]. None of our studies from 2007 to 2017 in the south of Benin confirmed that high prevalence of HR-HPV is associated to cervical lesions reported by another group in 2011 [24].

However, in the north of Benin the HR-HPVs mostly involved in cervical cancer were HPV39, 18, 45, 35 and in a lesser extend HR-HPV52, 33, 58 and 51 but no HR-HP16 was found [22]. In the context of HPV genotypes most encountered in asymptomatic women in the south of BENIN the nonavalent vaccine (Gardasil® 9) targeting HPV 6/11, 16, 18, 31, 33, 45, 52 and 58, may help to reduce HPV infection with these type of viruses but leaves out HPV51, HPV35, HPV66 and HPV68 also encountered in our aera. Regardless of technical sensitivity of the methodology used (nested versus real time PCR), the trend of HR-HPV genotypes remains low for HPV18 and HPV 16 which are predominant and associated to cervical cancer in developed countries. Vaccination can protect against HR-HPV-induced cervical cancer but not against natural occuring cervical cancer due to biomolecular alterations [18-22].

5. Conclusion

Overall, the presence of HR-HPV is not always relevant to predict cervical cancer development among African women investigated in the south of Benin. Considering the genotypes targeted with current vaccines and the fact that the immunization could barely cover all HPV infected women in our study, there is a crucial need to direct research towards molecular biomarkers for early screening and diagnosis of cervical cancer. Vaccination programs needs to target HR-HPV genotypes predominant in each country to guarantee full protection of population in the context of sub-Saharan countries.

Abbreviations

DNA: 2’ deoxynucleic acid; CUS: cervical uterine smears; HPV: Human Papilloma Virus; HR-HPV: high risk Human Papilloma Virus; ICUS: inflammatory cervical uterine smears; ND: not determined; PCR: polymerase chain reaction.

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Declarations

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The protocol of this study is approved by the Ethics Committee of the Institute of Biomedical Sciences and Applications (CER-ISBA) and by the ethic committee of the university of Parakou (Bénin). The protocol followed the guide lines of the Helsinki declarations.

Availability of data and materials

All data collected are included in this manuscript.

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