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Research Article

Molecular Epidemiology of High-Risk Human Papillomavirus in High-Grade Cervical Intraepithelial Neoplasia and in Cervical Cancer in Parakou, Republic of Benin

[1,2,3,7]T.M. Zohoncon, [1,2]T.C. Ouédraogo, [3,4]L.V.C. Brun, [8]D. Obiri-Yeboah, [1,2]W.F. Djigma, [5]S. Kabibou, [6]S. Ouattara, [3]M. Gomina, [1,2]A.T. Yonli, [1,2]V.J.T.E. Bazié, [2,6]C. Ouédraogo, [6]O. Lompo, [2,3]S.A. Akpona and [1,2,7]J. Simpore

[1]Laboratory of Molecular Biology and Genetics (LABIOGENE), University of Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]Pietro Annigoni Biomolecular Research Center (CERBA), 01 BP 364 Ouagadougou 01, Burkina Faso
[3]Teaching and Research Unit in Biochemistry and Molecular Biology, Faculty of Medicine, University of Parakou, BP 123 Parakou, Republic of Benin
[4]Teaching and Research Unit, Department of Anatomy and Cytopathology, Faculty of Medicine, University of Parakou, BP 123 Parakou, Republic of Benin
[5]Department of Service of Gynecology, University Hospital of Borgou and Alibori (CHUD-B/A), BP 123 Parakou, Republic of Benin
[6]Yalgado Ouédraogo University Hospital (CHU/YO), 03 BP 7022, Republic of Benin
[7]Faculty of Medicine, University of Saint Thomas d'Aquin, 06 BP 10212 Ouagadougou 01, Burkina Faso
[8]Department of Microbiology, School of Medical Sciences, University of Coast, University Post Office, Ghana

Abstract

Human papilloma virus (HPV) infection remains a worldwide concern, especially in Sub-Saharan Africa where cervical cancer is the leading cause of cancer death in women. The aim of the study was to determine the prevalence and genotypic distribution of High-Risk HPV (HR-HPV) involved in Cervical Intraepithelial Neoplasia (CIN) II and III and in cervical cancer in Parakou. Out of a total of 149 samples of cervical tissues archived, fixed and paraffin-embedded, 78 samples with histological diagnosis of CIN-II, CIN-III and cervical cancer went through deparaffinization with xylene, followed by an extraction of HPV DNA and the detection of HR-HPV by real-time multiplex PCR. The average age of the women was 40.05±13.99 years. The samples were positive to at least one HR-HPV genotype in 76.92% (50/65) of cases. The HR-HPV genotypes which are most common in the cervical cancer and in CIN-II and III were, respectively HPV-39 (38 and 37.50%), HPV-18 (35 and 31.30%), HPV-45 (35 and 31.30%), HPV-35 (9 and 25%) and HPV-52 (9 and 12.50%). The HPV-16 was absent. This study helped to detect (in samples archived, fixed and paraffin-embedded tissues) HR-HPV involved in high-grade precancerous lesions and in cervical cancer in Parakou, some of which are not covered by currently available vaccines.

Key words: HPV, CIN, cervical cancer, prevalence, real-time PCR

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Corresponding Author: Jacques Simpore, Pietro Annigoni Biomolecular Research Centre (CERBA)/LABIOGENE, University of Ouagadougou, 01 BP 364 Ouagadougou 01, Burkina Faso, Ouagadougou, Burkina Faso, West Africa Tel: +226 25361232 Fax: +226 25363242

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cervical cancer is the leading cause of cancer death in women in Sub-Saharan Africa. It has been established that cervical cancer is a virally-induced neoplasm and that the human papillomavirus (HPV) is the causative agent in 99% of cases (Ly, 2009). The HPVs are non-enveloped viruses of the Papillomaviridae family (De Villiers *et al*., 2004; Alain *et al*., 2010), whose High-Risk HPV (HR-HPV) analyzed in this study belong to the Alpha papillomavirus genus and to the human papillomavirus 5, 6, 7 and 9 species. The estimated incidence of cancers induced by HPV infection was 12.7 million in 2008 (Forman *et al*., 2012). However, there are ways of preventing HPV infection among which vaccination occupies a prominent place, as well as screening and treatment of precancerous lesions. In Africa, the prevalence of HPV infection reaches 21.3% with significant variations depending on regions; 21.5% in West Africa, 33.6% in East Africa and 21% in Southern Africa (WHO and ICO., 2009). Cervical cancer is the most common cancer in women in Sub-Saharan Africa. It is the second most common malignant tumor in women worldwide. Throughout the world, Invasive Cervical Cancer (ICC) is the cause of over 275,000 deaths per year of which 88% occur in countries with limited resources. In Sub-Saharan Africa, there are more than 75,000 new cases of ICC and over 50,000 deaths per year (Ferlay *et al*., 2010). In Benin, current estimates indicate an annual incidence of 925 cases of women with cervical cancer and 616 of them die (Piras *et al*., 2011). Cervical cancer screening through Pap test is not readily and most women are unaware of cervical cancer, its etiology and its prevention. Therefore, there is a real public health problem. The best mean of control remains vaccination against HPV. Based on literature and worldwide, especially in Europe and in some African countries, the most common genotypes are HPV-16 and HPV-18 (Denny *et al*., 2014; Heard *et al*., 2013; Guan *et al*., 2012), followed by HPV-31, 52 and 58 (Ogembo *et al*., 2015). HPV infection varies depending on regions. Some studies reported HR-HPV genotypes other than HPV-16 and 18 in Burkina Faso (Sagna *et al*., 2010; Djigma *et al*., 2011; Ouedraogo *et al*., 2011, 2015; Zohoncon *et al*., 2013).

The main objective of this study was to determine the prevalence and distribution of HR-HPV genotypes involved in high-grade Cervical Intraepithelial Neoplasia (CIN) and in cervical cancer from samples archived, fixed and paraffin-embedded tissues in the Departmental University Hospital of Borgou and Alibori (CHUD-B/A), Parakou, Republic of Benin.

MATERIALS AND METHODS

This was a descriptive and cross-sectional study with retrospective data collection.

Sample collection: The samples were collected in the Department of Anatomy and Cytopathology, CHUD-B/A which is the reference center in the northern part of the Republic of Benin. The study population consisted of archived, fixed and paraffin-embedded tissues. This was biopsy tissue samples and parts of hysterectomies and conizations patients in whom a histopathological diagnosis of high-grade Cervical Intraepithelial Neoplasia (CIN) or cervical cancer had been posed. The study focused on archived tissue blocks from January, 2009 to July, 2014. The paraffin blocks containing archived tissues have been sent to Ouagadougou (Burkina Faso) in the Department of Anatomy and Cytopathology, Yalgado Ouedraogo University Hospital (CHU/YO) where they went through microtome sections. Four tissue sections of 15 µm were cut for each eligible sample. Prior to the microtome cutting of the tissues, the blocks were placed at -20°C for 60 min. The resulting tissue sections were introduced into eppendorfs nuclease-free tubes after removing the excess paraffin. The samples collected were sent to the Laboratory of Molecular Biology and Genetics (CERBA/LABIOGENE, University of Ouagadougou, Burkina Faso) for molecular analysis.

DNA extraction: The HPV DNA was extracted using the commercial kit known as FFPE DNA Purification Product # 47400 (NORGEN BIOTEK CORPORATION, Canada). Before conducting the actual DNA extraction, the samples underwent dewaxing with xylene. The extraction was performed following the protocol provided by the manufacturer using micro-columns with collection tubes. The DNA thus extracted was stored at -20°C until amplification.

Detection of HR-HPV genotypes through real-time PCR: Amplification was done using the kit named HPV Genotypes 14 Real-TM Quant (SACACE Biotechnologies®, Italy) which is an *in vitro* real-time multiplex PCR test for the detection of 14 HR-HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). Each sample underwent multiplexed amplification in 4 tubes and each tube contained primers E6 and E7 target regions of three or four types of HR-HPV and human beta-globin gene as Internal Control (IC). For each sample, the 4 tubes, respectively corresponded to: 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 and 66. The amplification program was, 1 cycle of 95°C for 15 min, 5 cycles of 95°C for 5 sec followed by 60°C for 20 sec and 72°C for 15 sec and finally 40 cycles of 95°C for 5 sec followed by 60°C for 30 and 72°C for 15 sec. The results were interpreted with the Microsoft Excel program named "HPV Genotypes 14 Real-TM.xls" (SACACE Biotechnologies®, Italy) provided by the manufacturer.

Ethical considerations: This study received the approval (No. 31 of 31-12-2013) of the Research Ethics Committee of the Institute of Applied Biomedical Sciences (CER-ISBA), Republic of Benin and the approval (No. 2014-8-099 of 06-08-2014) of the Ethics Committee for Health Research (CERS), Burkina Faso. We complied with the confidentiality and anonymity condition with respect to information obtained from the various registers and the patient charts are kept strictly confidential.

Statistical analysis: Data were processed and analyzed on a microcomputer using SPSS 17.0, Epi Info 6. The Chi-square test was used for comparisons. The difference was statistically significant for $p<0.05$. The confidence interval was 95% and the prevalence of major diseases was calculated to determine data accuracy.

RESULTS

Among the samples of cervix received at the Department of Anatomy and Cytopathology CHUD-B/A from January, 2009 to July, 2014 and having undergone histological analysis, 149 cases of CIN and cervical cancer were recorded, of which 43.62% (65 cases) of cervical cancer and 56.38% (84 cases) of CIN. Out of the 149 samples, 78 samples diagnosed CIN-II, CIN-III and cervical cancer underwent PCR with the aim of detecting HR-HPV. The remaining 71 samples (diagnosed CIN-I) were excluded. Out of the 78 samples, 13 (16.66%) had invalid PCR result; 65 (83.34%) had valid PCR result, of which 50 samples were HR-HPV positive and 15 samples were negative (Table 1).

Characteristics of women and types of precancerous or cancerous cervical lesions: Women's age ranged from 18 to 88 years with an average age of 40.05±13.99 years. The most represented age group was that of women aged 30-39 years (35.57%) followed by the age group of 40-49 (22.15%), 20-29 (20.13%), 50-59 (10.07%), 60-69 (8.05%), 80-89 (2.01%), 70-79 (1.34%) and 15-19 years (0.67%). Among the samples with histological diagnosis of ICC, 55/65 or 84.62% (95% CI 73.52-92.38) were Squamous Cell Carcinomas (SCC), 8/65 or 12.31% (95% CI: 05.47-22.82) Adenocar Cinomas (AC) and 2/65 or 3.08% (95% CI: 0.37-10.68) adeno-squamous carcinoma (ASC). Table 2 shows the various histological diagnoses of precancerous and cancerous cervical lesions. Table 3 shows the main histological results based on the age groups of the women.

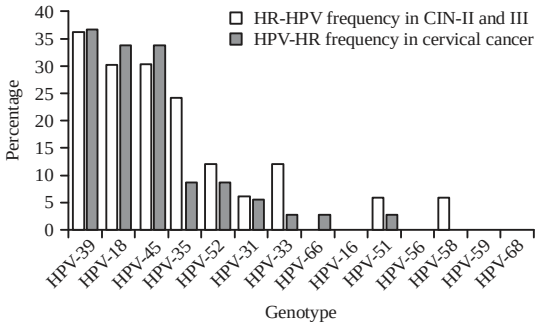

Fig. 1: Frequency of HR-HPV genotypes in cervical cancer and in CIN-II and III, Parakou, 2009-2014

Detection of HR-HPV genotypes and their frequencies: Among the 65 appropriate PCR results, 50/65 or 76.92% (95% CI: 64.52-86.10) of the samples were positive to at least one HR-HPV genotype. Genotypes of the 14 HR-HPV sought in the samples, 10 were identified, namely HPV-39, 18, 45, 35, 52, 31, 33, 51, 58 and 66 in different proportions. The HR-HPV genotypes most common in cervical cancer and in the high-grade CIN were HPV-39 with respective frequencies of 38 and 37.50%, followed, respectively, by HPV-18 (35 and 31.30%), HPV-45 (35 and 31.30%), HPV-35 (9 and 25%) and HPV-52 (9 and 12.50%). Genotypes of HPV-16, 56, 59 and 68 were not detected in the samples tested. The HPV-66 genotype was identified in a case of squamous cell carcinoma. Figure 1 shows the distributions of HR-HPV genotypes in cervical cancer and in the high-grade CIN.

Multiple or isolated HR-HPV infections: The number of HR-HPV genotypes per infected woman ranged from 1-3. Multiple HR-HPV infections were found in 36% of cases; while, isolated infection was found in 64% of the cases. Among the cases of isolated or multiple HR-HPV infections, the isolated HPV-39 genotype infection was the most common (20%) with, respectively 16% in the cases of cervical cancer and 4% in the cases of high-grade CIN (Table 4).

Table 1: Detection of the human beta-globin gene through PCR of HR-HPV in high-grade cervical intraepithelial neoplasia and in cervical cancer, Parakou, 2009-2014

	PCR HR-HPV				
β-globin gene	Positive	Negative	Inappropriate	Total	p-value
Present	50	15	0	65	0.001
Absent	0	0	13	13	
Total	50	15	13	78	

Negative PCR (HR-HPV undetected; β-globin detected); Inappropriate PCR (neither HR-HPV detected nor β-globin detected); Positive PCR (HR-HPV detected), HR-HPV (high-risk HPV)

Table 2: Histological diagnosis of precancerous and cancerous cervical lesions, Parakou, 2009-2014

Parameters	Histological diagnosis of cervical lesions	Number (%)	95% IC
*Adenocarcinoma	*In situ* Adenocarcinoma	07 (04.70)	(01.90-09.44)
	Villoglandular papillary adenocarcinoma	01 (00.67)	(00.00-03.68)
*Adeno-squamous carcinoma	Invasive adeno-squamous carcinoma	02 (01.34)	(00.16-04.76)
	Keratinizing, invasive and well-differentiated squamous cell carcinoma	22 (14.77)	(09.49-21.50)
	Non-keratinizing, invasive and well-differentiated squamous cell carcinoma	21 (14.09)	(08.94-20.73)
*Squamous cell carcinoma	Invasive and moderately differentiated squamous cell carcinoma	07 (04.70)	(01.90-09.44)
	Invasive and poorly differentiated squamous cell carcinoma	01 (00.67)	(00.00-03.68)
	Micro invasive squamous cell carcinoma	04 (02.69)	(00.74-06.73)
*CIN	CIN-I	52 (34.90)	(27.28-43.13)
	CIN-II	20 (13.42)	(08.40-19.97)
	CIN-III	12 (08.05)	(04.23-13.65)
Total		149 (100.0)	

*p-value<0.001

Table 3: Type of precancerous and cancerous cervical lesions based on the age groups of the women, Parakou, 2009-2014

	Cervical cancer			CIN			
Age group (years)	Squamous cell carcinoma	Adeno-carcinoma	Adeno squamous carcinoma	CIN-I	CIN-II	CIN-III	Total
15-19	0	0	0	0	1	0	1
20-29	3	2	0	20	3	2	30
30-39	18	2	0	17	9	7	53
40-49	10	3	0	9	5	3	33
50-59	9	1	2	2	1	0	15
60-69	9	0	0	2	1	0	12
70-79	2	0	0	0	0	0	2
80-89	3	0	0	0	0	0	3
Total	55	8	2	52	20	12	149

DISCUSSION

Molecular biology techniques are used for the investigation of several diseases including cervical cancer which is the subject of this study. Archived, formalin-fixed and paraffin-embedded tissues with a histopathological diagnosis of cervical cancer and cervical intraepithelial neoplasia are used for retrospective molecular epidemiological studies on HPVs. The main limitation of this study is the size of the high-grade CIN and cervical cancer samples (78) which is not large enough. The real-time PCR technique used with the kit named HPV genotypes 14 Real-TM Quant (SACACE biotechnologies®, Italy) is a fine tool for molecular diagnostic capable of detecting fourteen HR-HPV genotypes for etiological research of precancerous and cancerous cervical lesions. It is an *in vitro* amplification test which specificity and sensitivity are 100% each. The average age of the women in the study was 40.05±13.99 years. This result is close to those reported in other studies, 54.1±7.6 years in Ghana (Attoh *et al*., 2010), 51.9±12.3 years in Mali and Senegal (Ndiaye *et al*., 2012), 44.3±8.2 years in Congo (Boumba *et al*., 2014) and 46.4 years in Thailand (Supho *et al*., 2014). The standard deviation was not specified in the study of Supho *et al*. (2014) in Thailand. In this study, 16.66% (13/78) of the PCR were invalid because the β-globin was absent as well as the HPV detection. Missaoui *et al*. (2010) also using formalin-fixed and paraffin-embedded tissues, had been reported that the β-globin negative in 25/146 was (17.1%) samples considered as non-contributory. This result is similar to 16.66% (p = 0.931), although in the study of Missaoui *et al*. (2010) histological lesions have been the subject of a double reading by two anatomical pathologists. Similarly, with the use of xylene for deparaffinization, Steinau *et al*. (2011) had found similar results (p = 0.622), 19.33% (29/150). In the same vein,

Table 4: Multiple or isolated HR-HPV infections in high-grade precancerous lesions and in cervical cancer, Parakou, 2009-2014

HR-HPV genotypes	Total No. (%)	Cervical cancer No. (%)	CIN-II and III No. (%)	p-value
HPV-39	10 (20)	8 (16)	2 (4)	NS
HPV-18	8 (16)	6 (12)	2 (4)	NS
HPV-45	5 (10)	3 (6)	2 (4)	NS
HPV-35	4 (8)	2 (4)	2 (4)	NS
HPV-18, 45	4 (8)	4 (8)	0 (0)	NS
HPV-18, 39	2 (4)	1 (2)	1 (2)	NS
HPV-18, 39, 45	2 (4)	1(2)	1 (2)	NS
HPV-39, 45	2 (4)	2 (4)	0 (0)	NS
HPV-45, 52	1 (2)	1 (2)	0 (0)	NS
HPV-45, 51, 52	1 (2)	1 (2)	0 (0)	NS
HPV-52	2 (4)	1 (2)	1 (2)	NS
HPV-18, 39, 52	1 (2)	0 (0)	1 (2)	NS
HPV-31	1 (2)	1 (2)	0 (0)	NS
HPV-31, 35	1 (2)	1 (2)	0 (0)	NS
HPV-31, 39, 45	1 (2)	0 (0)	1 (2)	NS
HPV-33	1 (2)	0 (0)	1 (2)	NS
HPV-33, 35, 58	1 (2)	0 (0)	1 (2)	NS
HPV-33, 39	1 (2)	1 (2)	0 (0)	NS
HPV-35, 45, 51	1 (2)	0 (0)	1 (2)	NS
HPV-66	1 (2)	1 (2)	0 (0)	NS
TOTAL	50 (100)	34 (68)	16 (32)	

NS: Not significant

Bateman *et al*. (2015) had found that the method of extraction using xylene gave low-quality results compared to that using heat and 88% of these results (100/114 case of cervical cancer) were valid with PCR. This possibility of having non-contributory samples could be due to the degradation of the DNA of archived formalin-fixed tissues. Indeed, it is known that formalin can alter the structure of nucleic acids, so that the result of the extraction from formalin-fixed and paraffin-embedded samples may be of low quantity and low quality (Gouveia *et al*., 2014). Paraffin is like a physical barrier, DNA cross-linking and PCR inhibitor. Although, the DNA of archived tissue is generally preserved over long periods of time, its fragmentation and its protein cross-linking by formaldehyde exposure, as well as the presence of paraffin, could have significant adverse effects on the DNA yield and the amplification efficiency (Steinau *et al*., 2011). Internal control (human β-globin gene) may not be detected in the event of excess paraffin or an insufficient quantity of epithelial cells. The DNA extraction using heat (without xylene) would be more successful than that based on the use of xylene. The inappropriate results in this study may also be explained by the presence of other HPV genotypes that cannot be detected by the amplification kit used, which only allowed the detection of 14 HR-HPV genotypes.

However, the HR-HPV detection rate in this study was 76.92% (50/65). This result corroborates respectively ($p = 0.919$ and 0.614) the HPV detection rates of 77.59 and 73.60% reported by Steinau *et al*. (2011) in Georgia and Missaoui *et al*. (2010) in Tunisia, in formalin-fixed and paraffin-embedded tissues. Although, these authors have sought high-risk and low-risk HPV, there is no statistically significant difference between the results. In Ghana, Attoh *et al*. (2010) had reported 98% (49/50) of HPV infection in the case of cervical cancer. This high rate of HPV detection in cervical cancer could be explained by the fact that the tissue sections used for the PCR were previously checked with a microscope to ensure that they actually contain tumor cells. Similarly, Ndiaye *et al*. (2012) had reported HPV detection rates at 88.1% (28/32) and 86.5% (110/132), respectively in Mali and Senegal. These relatively high rates of HPV detection could be explained by an appropriate technique for tissue blocks conservation, the double histological reading, the verification of the presence and the quantification of necrotic tumor, the percentage of tumor in the tissue section and therefore the sample adequacy for a possible HPV DNA test.

The high-grade CIN and cervical cancer were more frequent in the age group of 30-39 years (35.57%) followed by the age group of 40-49 (22.15%), 20-29 (20.13%) and 50-59 years (10.07%). These results are similar to those reported by Siddiqa *et al*. (2014) in Pakistan who had found a high incidence of cervical cancer in women aged 41-50 years followed by the age groups of 31-40 and 50-60 years.

The frequency of cervical cancer in this study (43.62% or 65/149) is greater ($p<0.001$) than 16.71% (133/796) reported by Lesnikova *et al*. (2009) in Denmark and lower ($p = 0.013$) than 61.10% (47/77) reported by Siddiqa *et al*. (2014) in Pakistan, all from archived and paraffin-fixed tissues. This significant difference could be explained by the variation in

the sample size and the techniques used. Whatever the studies, among the cases of invasive cervical cancer, squamous cell carcinomas are more common than adenocarcinomas and adeno-squamous carcinoma as observed in this study ($p<0.001$). In this study, squamous cell carcinoma accounted for 84.62% (55/65) of the cases of cervical cancer; adenocarcinoma accounted for 12.31% (8/65) and adeno-squamous carcinoma represented 3.08% (2/65). Other authors had reported similar results ($p<0.05$): Lesnikova *et al.* (2009) had reported 79% of cases of squamous cell carcinomas and 21.05% of adenocarcinomas cases; Siddiqa *et al.* (2014) had identified among the cervical cancer cases, 91.49% of squamous cell carcinomas cases, 2.12% of adenocarcinomas and 6.38% of adeno-squamous carcinoma, Pirek *et al.* (2015) in Cameroon had reported 91.7% of squamous cell carcinomas and 6.6% of adenocarcinomas.

Genotypes of HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 73 are known as carcinogenic (Hausen, 2002). In this study, HPV-39, 18, 45, 35, 52, 31, 33, 51, 58 and 66 were detected with decreasing frequency in the cervical cancer and high-grade CIN. HR-HPV genotypes most common in cervical cancer and CIN-II and III were HPV-39 with respective frequencies of 38 and 37.50%; followed, respectively HPV-18 (35 and 31.30%) and HPV-45 (35 and 31.30%) and HPV-35 (9 and 25%). These genotypes are close to those found in some African countries where the most frequent genotypes were HPV-16, HPV-18, HPV-35 and HPV-45 (Denny *et al.*, 2014; Guan *et al.*, 2012) with the exception that HPV-16 was not detected in this study.

In Pakistan, Gul *et al.* (2015) had reported a prevalence of 32.8% for HPV-18 in cervical cancer; this result is similar to the 35% found in this study. Gul *et al.* (2015) had found that HPV-16 was the most common, with a prevalence of 44.8% in cervical cancer unlike this study in which HPV-16 was not detected in either cervical cancer or in CIN II and III. Other authors have also reported the prevalence of HPV-16, HPV-18 and HPV-45 in the case of cervical cancer: 32.69% of HPV-16, 34.62% of HPV-18 and 25% of HPV-45 (Haghshenas *et al.*, 2013), 52.5% of HPV-16 and 11.8% of HPV-18 (Boumba *et al.*, 2014), 15.4% of HPV-16 in a group of women of African origin (Tornesello *et al.*, 2014). Abate *et al.* (2013) had found that HPV-16 was the most frequent genotype identified in paraffin embedded cervical tissue samples from Ethiopia (91%, 136/149) and the Sudan (82.5%, 66/80) followed by HPV-52, 58, 18 in Ethiopia and HPV-18, 45, 52 in Sudan.

In Benin, Baba-Moussa *et al.* (2006) by comparing the results of the cytological analysis (Pap test) to those of the conventional PCR (PCR products subjected to agarose gel electrophoresis) had reported that over the 99 smears tested, 88 were PCR-positive with consensus primers GP5+/GP6+ and HPV types determined by the use of specific primer were HPV 33 (56%), followed by HPV 16 (18.2%), HPV 6/11 (18%) and HPV 18 (17%). These different results may be explained by the fact that the sample types and the amplification techniques are not the same.

In the literature, the HR-HPV, mostly type 16 and 18, are recognized as the main root causes of invasive cervical cancer and its precursor lesions (Zur Hausen, 1996; Hausen, 2002). Several meta-analyzes have confirmed that the five HR-HPV of human beings which are most common in women with and without neoplastic diseases of the cervix are HPV-16, 18, 31, 52, and 58 (Ogembo *et al.*, 2015). However, the HPV-39 genotype is dominant in this study, followed by HPV-18 and HPV-45 irrespective of the histopathological type of the cervical cancer. They are followed by HPV-35, HPV-52, HPV-33, HPV-51 and HPV-58 in CIN-II and III while they are followed by HPV-35, HPV-52, HPV-31, HPV -33, HPV-51 and HPV-66 in the case of cervical cancer.

The HPV-39 genotype was also detected in other studies among the most frequent HPV. In South Africa, Lebelo *et al.* (2015) had reported that HPV-39 (18.7%) was among the most common genotypes archived, fixed and paraffin-embedded tissues; they also detected HPV-18, HPV-16 and HPV-56. But they also noted as in this study, the absence of HPV-68. Boumba *et al.* (2014) had also detected genotypes of HPV-31, HPV-33 and HPV-35 in invasive cervical cancer in addition to HPV-16 and -18.

Genotypes of HPV-51, HPV-58 and HPV-66 were less frequent in this study. On the contrary, HPV-58 was one of the most common genotypes (HPV-16, -52 and -58) in a province of China in asymptomatic women (Xue *et al.*, 2015). Similarly, in the North-East of China, HPV-58 and HPV-16 were the most common (Sun *et al.*, 2014). The only HPV-66 genotypes detected in a case of cervical cancer in this study was also reported in another study conducted in adolescents in Belgium where the most frequent genotypes were HPV-16 (16.7%), HPV-51 (14.6%), HPV-66 (10.4%), HPV-31 (9.9%) and \HPV-39 (9.1%) (Merckx *et al.*, 2014). Another study conducted by Attoh *et al.* (2010) in Ghana had reported a low prevalence of 2% of HPV-66 infection, similar to that of this study.

HPV infection may be isolated or multiple. Indeed, in this study, the number of genotypes per infected woman ranged from 1-3 with 36% of multiple infections. Tornesello *et al.* (2014) had reported a multiple infection rate of 33.3% among women of African origin; this result is similar to the 36% reported in the study, while, Lebelo *et al.* (2015) had reported that the majority of South African women with squamous cell

carcinoma of the cervix had multiple HPV infection. However, Attoh *et al*. (2010) had reported 26% of multiple infections in cases of cervical cancer in Ghana. This variation could be due to the status of the Human Immunodeficiency Virus (HIV) of South African women. Indeed, HIV infection is a risk factor of precancerous lesions of the cervix and also probably for invasive cervical cancer (Sahasrabuddhe *et al*., 2007). Patients with HIV are more sensitive to multiple HPV infections.

The distribution of high-risk HPV varies depending on geographic and demographic factors. This change in the geographic distribution of HPV genotypes might influence the effectiveness of vaccination (Clifford *et al*., 2003). It is generally accepted that the immune response to virus-like-particles (VLPs) of HPV is specific to this type and therefore, protection through vaccination should be limited to the types included in the vaccine (Delvenne, 2006). However, an *in vitro* observation showed a certain cross-neutralization level between types 16 and 31 and between types 18 and 45.

CONCLUSION

The HPV 39 genotype is at the forefront of HR-HPV genotypes involved in high-grade CIN and in cervical cancer in Parakou, followed by the genotypes of HPV-18, HPV-45, HPV-35 and HPV 52. The HPV 16 genotype was not detected. The significance of HPV-39, 45, 35 infections in this study shows the need for a large-scale study for a better mapping of the HR-HPV that could guide research to other multivalent vaccines.

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