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RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPES INVOLVED IN INVASIVE CERVICAL CANCER FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES IN OUAGADOUGOU, BURKINA FASO

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ABSTRACT

Background: Cervical cancer (CC) is a global public health issue. It is due to the persistence of infection by high-risk Human Papillomavirus (HR-HPV). Recently, Sub-Saharan Africa has the highest death rate caused by CC. The objective of this study was to detect HR-HPV genotypes involved in cases of invasive CC histologically confirmed in Ouagadougou.

Methods: A total of 112 formalin-fixed and paraffin-embedded-blocks of cervical tissue diagnosed in the department of pathological anatomy and cytology of the 'Yalgado Ouédraogo' University Hospital and archived between 2009 and 2015 were included in the study. Fourteen HR-HPV genotypes were tested for by multiplex-real-time-PCR within the invasive CC tissue blocks.

Results: Of the fourteen genotypes tested for, eleven were identified. The prevalence of HR-HPV infection was 72.31% (47/65). The most common genotypes in invasive CC in Ouagadougou were: HPV18(25.71%), HPV31(15.71%), HPV39(12.86%), HPV16(12.86%), HPV45(12.86%), HPV35(7.14%) and HPV58(5.71%).

Findings: In this study, HPV-18; 31; 39; 16 and 45 were the HR-HPV genotypes most involved in invasive CC in Ouagadougou. These genotypes identified are not all covered by the HPV vaccines available. There is therefore a need for making a mapping of all HPV circulating in the West African region for possible development of new HPV vaccines.

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INTRODUCTION

Worldwide, invasive cervical cancer is responsible for over 275,000 deaths each year, with 88% occurring in countries with limited resources. Cervical cancer is the leading cause of cancer death among women in Africa. In Sub-Saharan Africa, there are more than 75,000 new cases of invasive cervical cancer and over 50,000 deaths per year (Ferlay *et al*., 2010). In Burkina Faso, estimates indicate that 1,230 women are diagnosed with cervical cancer each year with 838 women dying of it (www.who.int/hpvcentre.2010. (Accessed 04.12.15)). Because of its high-risk genotypes, HPV is closely associated with cervical cancer. Currently, more than 120 HPV genotypes have been identified with about forty infecting the anogenital tract. Of this number, twenty genotypes are classified as oncogenic. Overall, most of the studies on the distribution and prevalence of high-risk HPV (HR-HPV) in cervical cancer indicate a predominance of HPV 16 and 18

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(Ly, 2009). However, in some countries, there is a low prevalence of those genotypes in cervical cancer; they are preceded by other genotypes (de Sanjose *et al*., 2007; Bosch *et al*., 2008). In Burkina Faso, no study has yet focused on the distribution and prevalence of HR-HPV in cases of invasive cervical cancer confirmed histologically. Studies conducted so far focused only on cervical cell samples from women with unknown pathology status and/or HIV (human immunodeficiency virus) positive status. Those studies indicated a predominance of HPV 35, 52, 31 and 39 (Djigma *et al*., 2011; Zohoncon *et al*., 2013; Ouedraogo *et al*., 2015; Traore *et al*., 2016a; Traore *et al*., 2016b). In addition, a recent study on the HR-HPV involved in cases of intraepithelial cervical neoplasia in Ouagadougou showed a prevalence of HPV 39, 35 and 45 (Ouédraogo *et al*., 2016). Based on these findings, we assumed a possible emergence of HR-HPV genotypes other than HPV 16 and 18. Therefore, the objective of this study was to determine the distribution of 14 HR-HPV genotypes in cases of invasive cervical cancer in Ouagadougou (Burkina Faso).

MATERIALS AND METHODS

Sampling and Data Collection

We conducted a descriptive cross-sectional study with retrospective data collection. A total of 112 archived formalin-fixed and paraffin-embedded cervical tissue blocks dated 2009-2015, with histological diagnosis of invasive cervical cancer were collected using records of the pathological anatomy and cytology laboratory of the 'Yalgado Ouédraogo' University Hospital (CHU-YO) in Ouagadougou (Burkina Faso). Tissue blocks were sectioned with a microtome and 5 sheets of not more than 20 microns from the section of each sample were introduced in *eppendorfs nuclease-free* tubes after reducing the excess paraffin and forwarded to the Laboratory of Molecular Biology and Genetics (LABIOGENE), University Ouaga I Professor Joseph Ki-Zerbo, for molecular analysis.

Extraction and Detection of HR-HPV by Multiplex Real-time PCR

The extraction of the DNA was done using the FFPE DNA Purification kit (Norgen Biotek Corporation, Italy) following the manufacturer's protocol. This kit allows a dewaxing with xylene followed by rehydration with absolute ethanol and finally the actual DNA extraction.

The detection of HR-HPV genotypes was done using Sacycler-96 Real time PCR (Sacace Biotechnologies, Como, Italy) and HPV genotypes 14 Real-TM Quant (Sacace Biotechnologies, Como, Italy) allowing multiplex detection of 14 high-risk HPV genotypes.

Ethical Considerations

Our study was approved by the Ethics Committee for Health Research (CERS) in Burkina Faso, by deliberation No. 2014-8-099. We complied with confidentiality and anonymity with respect to information collected.

Statistical Analysis

Statistical analyzes were performed using the IBM SPSS statistics 20 software and the chi-square test was used to compare the results. Results were considered significant for a p-value less than 5%.

RESULTS

Of the 112 formalin-fixed and paraffin-embedded blocks of cervical tissue that were diagnosed with invasive cervical cancer, the average age of the women was 46.32 ± 12.76 years (21-84 years). Table 1 shows the histological types of cervical cancer depending on age groups. Squamous cell carcinomas (SCC) were more common than adenocarcinomas (ADC). In 55.35% of cases, invasive cervical cancer had occurred among women aged 35 to 54. After molecular characterization of the 112 samples, 58.04% (65/112) had a valid result (beta globin detected) and 41.96% (47/112) of the samples had an invalid result (beta globin not detected). Among the valid results, 47 samples (72.31%, 47/65) were HPV positive and 18 samples (27.69%; 18/65) were HPV negative. Overall, 11 genotypes of the 14 sought were identified with prevalence varying between 25.71% and 1.43% (Table 2). Among the genotypes found, the most common, in descending order, were HPV18 (25.71%), HPV31 (15.71%), HPV39 (12.86%), HPV16 (12.86%) and HPV45 (12.86%), HPV35 (7.14%) and HPV58 (5.71%) (Table 2). Among the HR-HPV positive samples, we detected 65.96% (31/47) of isolated infections and 34.04% (16/47) of multiple infections with a number of genotypes ranging from 1 to 4 per individual and an average of 1.56 ± 0.13. HPV18 was the most common both in multiple infections (50%; 8/16) and isolated infections (36%; 9/25). In the multiple infections, HPV18 was associated with 6 other genotypes against 3 in HPV16 (Table 3). Considering the multiple and isolated infections, the accumulated number of genotypes was 70. HPV 16 and 18 were detected in 38.57% (27/70) of HR-HPV and the other HR-HPV genotypes (HPV31, 35, 39, 45, 51, 52, 56, 58 and 59) represented 61.43% (43/70). The HPV other than HPV 16 and 18 namely HPV31, 35, 39, 45, 51, 52, 56, 58 and 59 were therefore more common in both squamous cell carcinomas and adenocarcinomas.

Table 1. Histological types of invasive cervical cancer cases according to age groups

Age group (years)	Squamous cell carcinomas (SCC)		Adenocarcinomas (ADC)		Total	P value
	N (%)	IC 95%	N (%)	IC 95%	N (%)	
≤ 24	02 (01.79)	(0.22-6.30)	00 (00.00)	(0.00-0.00)	02 (01.79)	
25 to 34	15 (13.39)	(7.69-21.13)	02 (01.79)	(0.22-6.30)	17 (15.18)	
35 to 44	37 (33.03)	(24.43-42.56)	00 (00.00)	(0.00-0.00)	37 (33.03)	0.106
45 to 54	22 (19.64)	(12.74-28.22)	03 (02.68)	(0.55-7.63)	25 (22.32)	
≥ 55	25 (22.32)	(12.74-28.22)	06 (05.36)	(1.99-11.30)	31 (27.68)	
Total	101 (90.18)	(83.11-94.99)	11 (09.82)	(5.01-16.89)	112 (100.00)	

HPV16 was not found in adenocarcinomas. Table 4 shows the prevalence of HR-HPV genotypes in histological types of cervical cancer by considering the total accumulated genotypes. Table 5 shows the distribution of genotypes according to age groups (Table 5). The rate of cancer induced by HPV was the highest in the 35-54 age group.

Table 2. Prevalence of HR-HPV genotypes

HR-HPV Genotypes	Numbers N (%)	95%IC
HPV18	18 (25.71)	(16.01-37.56)
HPV31	11 (15.71)	(08.11-26.38)
HPV39	09 (12.86)	(06.05-23.01)
HPV45	09 (12.86)	(06.05-23.01)
HPV16	09 (12.86)	(06.05-23.01)
HPV35	05 (07.14)	(02.36-15.89)
HPV58	04 (05.71)	(01.58-13.99)
HPV52	02 (02.86)	(00.35-09.85)
HPV51	01 (01.43)	(00.04-07.70)
HPV56	01 (01.43)	(00.04-07.70)
HPV59	01 (01.43)	(00.04-07.70)
HPV33	00 (00.00)	(00.00-00.00)
HPV66	00 (00.00)	(00.00-00.00)
HPV68	00 (00.00)	(00.00-00.00)
Total	70	100

Table 3. Different associations in multiple infections

HR-HPV Genotypes	Numbers N (%)
16, 31	01 (06.25)
16, 31, 39	01 (06.25)
16, 31, 39, 58	01 (06.25)
18, 31, 45, 58	01 (06.25)
18, 35	01 (06.25)
18, 39	02 (12.50)
18, 45	02 (12.50)
18, 51	01 (06.25)
18, 58	01 (06.25)
31, 39	04 (25.00)
31, 39, 45, 52	01 (06.25)
Total	16

HPV18 was the most common in the age group greater or equal to 45 years, while HPV16 was common in the age group of 25-44 years (Table 5).

made with an excess of paraffin. To confirm the negative impact of paraffin, Steinau *et al.* had compared the dewaxing method used in our study with another method using heat (Steinau *et al*., 2011). He obtained better results with the thermal method. The most common genotypes in descending order were the following: HPV18 (25.71%), HPV31 (15.71%), HPV39 (12.86%), HPV16 (12.86%) HPV16 (12.86%), HPV45 (12.86%), HPV35 (7.14%) and HPV58 (5.71%). Now, according to the International Agency for Research on Cancer, the most common HR-HPV genotypes associated with cancer are HPV 16 (53%), HPV18 (15%), HPV45 (9%), HPV31 (6%), and HPV33 (3%) (IRAC, 2000). In Africa, the distribution and prevalence of HPV genotypes seem different from those observed in the world and we also see that in Africa, this situation is disparate across countries (De Vuyst *et al*., 2003; Louie *et al*., 2009; Tamegao-Lopes *et al*., 2014. In Burkina Faso as well as in Benin, genotyping on endocervical and tissue samples already confirmed this disparity with preponderance of HR-HPV other than HPV 16 and 18 (Ouedraogo *et al*., 2015; Traore *et al*., 2016a; Traore *et al.,* 2016b; Ouedraogo *et al*., 2011; Zohoncon *et al*., 2016).

In this study, it is worth noting a smaller difference between the prevalence of the most predominant genotypes and those of IARC. Indeed, while according to IARC the cumulative prevalence of the first two genotypes (HPV16-18) was 68%, we found in this study the cumulative frequency of the two (HPV18 and 31) and three (HPV18, 31 and 39) first genotypes which were respectively 41% and 53%. Constant in all studies conducted in Burkina Faso is the low frequency of HPV16 and the predominance of 'HPV-30' and 'HPV50' family. HPV16 and 18 were detected in 38.57% (27/70) of samples and the other HR-HPV (HPV31, 35, 39, 45, 51, 52, 56, 58 and 59) were found in 61.43% (43/70) of samples. HPV other than HPV 16 and 18 namely HPV31, 35, 39, 45, 51, 52, 56, 58 and 59 were more common in both squamous cell carcinomas and

Table 4. Prevalence of HR-HPV genotypes in histological types of invasive cervical cancer

Histological Type	HR-HPV Genotypes			Total
	16	18	Others*	
Squamous cell carcinoma	09 (12.85%)	15 (21.43%)	36 (51.43%)	60
Adenocarcinoma	00 (00.00%)	03 (04.29%)	07 (10.00%)	10
Total	09	18	43	70

Others* = HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

Table 5. Distribution of HR-HPV genotypes according to age groups

Age group	HR-HPV Genotypes			Total
	16	18	Others*	
25-34	02 (02.86%)	02 (02.86%)	07 (10.00%)	11
35-44	05 (07.14%)	03 (04.28%)	12 (17.14%)	20
45-54	00 (00.00%)	08 (11.43%)	12 (17.14%)	20
≥55	02 (02.86%)	05 (07.14%)	12 (17.14%)	19
Total	9	18	43	70

Others* = HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

DISCUSSION

This study identified HR-HPV genotypes in archived formalin-fixed and paraffin-embedded samples. Considering the valid results, the prevalence of HR-HPV was 72.30% (47/65). The positivity rate is relatively low compared to other studies. In Pakistan, Gul and Sidiqua have respectively found HPV positivity prevalence rates of 88% and 100% in cervical lesions from paraffin-embedded tissue blocks (Siddiqa *et al*., 2014; Gul *et al*., 2015). For paraffin-embedded samples, dewaxing is an important step especially when the cuts are

adenocarcinomas. Most infections were isolated and reflected by the average of 1.56 ± 0.13 genotypes per individual. Several studies have confirmed the high rate of isolated infections in cervical cancer (Naucler *et al*., 2004; Jacquard *et al*., 2010). Isolated infections due to HPV31, 35, 39, 45, 51, 52, 56, 58 and 59 were 64.51% (20/31) in all isolated infections and 42.55% (20/47) of all samples with positive results. This frequency would mean that for every 100 cervical cancers confirmed by a molecular test (PCR), at least 42 of those cancers would be solely due to these genotypes. In this study, of a combination of 70 genotypes found, 61.43% of those

genotypes were HR-HPV31, 35, 39, 45, 51, 52, 56, 58 and 59 and that alone were responsible for 42.55% of cancers without neglecting their action in cases of co-infections. HPV18 was the most common in multiple infections (50%; 8/16) and in the multiple infections HPV18 was associated with 6 other genotypes against 3 in HPV16. The average age of patients in our study was 46.36 ± 12.94 years, ranging from 21 to 84, and a peak between 35 and 54 years. This result is close to that of N'Guessan *et al.* in Côte d'Ivoire who found an average age of 48.5 years ranging from 30 to 81 years, and a peak between 41 and 50 years (N'guessan *et al*., 2009). The high prevalence of cervical cancer in this age group (35 and 54) which is relatively early seems to be related to the increase in risk factors such as poor socioeconomic conditions, early sexual activity, multiple sexual partners, thus increasing the risk of exposure to HPV and other sexually transmitted infections (Banza *et al*., 1999). In developing countries, HPV infection increases from the age of 25 and decreases between the age of 45 and 54 (de Sanjose *et al*., 2007). This early infection in these countries is related to risk factors such as early marriage, early sex, poverty, multiple sexual partners (Singh *et al*., 2012; Khan *et al*., 2007). These factors that induce and maintain early HPV infection, combined with its natural history likely explain the high prevalence of cancer in the 35-54 age group. Despite the shortcomings related to the size and quality of some samples, this study provides interesting information that can be used in cervical cancer prevention.

Indeed, in this study, the prevalence of HR-HPV non-covered by the two existing vaccines indicates a possible emergence of these genotypes. Studies in other parts of the world also indicate a significant frequency of these genotypes. In Africa and North America, HPV16 was respectively followed by HPV52, 58 and HPV52, 53 (de Sanjose *et al*., 2007; Bosch *et al.*, 2008). Specifically in Nigeria, HPV16 was followed by HPV31 and 35 (Thomas *et al*., 2004). All these figures clearly demonstrate the significance of HR-HPV other than HPV 16 and 18 in the occurrence of cancer and particularly in Africa. This situation raises the issue of the lack of preventive action of the two existing vaccines. The cost of vaccines and the lack of data is a major obstacle in the prevention of HPV infection and cervical cancer control. Therefore, there is the need for conducting large-scale studies to confirm the emergence of these genotypes for the development of vaccine covering more than two HR-HPV genotypes. Since 2014, a nanovalent vaccine has been developed. In addition to the four genotypes (16, 18, 6 and 11), this vaccine targets genotypes 31, 33, 45, 52 and 58. This vaccine which is directed against 9 HPV genotypes is being evaluated for an authorization for sale on the Western market (WHO, 2014). However, this nanovalent vaccine still does not cover all genotypes identified in this study.

Conclusion

Of the fourteen HR-HPV genotypes tested for, eleven were found. The most common genotypes in invasive cervical cancer among women in Ouagadougou are HPV18, HPV31, HPV39, HPV45, HPV16, HPV35 and HPV58. We found that the ''HPV 30' family and HPV 45 family is more predominant than HPV 16, while they are not all covered by the HPV vaccines currently available. In cases of invasive cervical cancer, HPV31, 35, 39, 45, 51, 52, 56, 58 and 59 were found in 61.43%. These data need to be confirmed by expanding the sampling and using other extraction techniques to improve DNA yield in archived formalin-fixed and paraffin-embedded cervical tissues.

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Declaration of interest

The authors declare that there is no potential conflict of interest.

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