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# Human Papillomavirus High-Risk Molecular Identification Among Senegalese Women

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## Abstract

**Background:** Human Papillomavirus and cervical cancer have become a major preoccupation for the medical and scientific international communities. Human Papillomavirus leads to precancerous lesions that can evolve in cervical cancer. Senegal is ranked 15th in the world for the incidence of cervical cancer. According to WHO, cervical cancer represented 30% of all deaths by cancer among women, representing the most frequent death by cancer in Senegal, followed by breast cancer (15.5%). The most frequent types of HPV found in cervical cancer are 16, 18, 31, 33, 35, 45, 52 and 58.

**Objectives:** The objective of our study is to evaluate, using molecular methods, the prevalence of HPV among Senegalese women and identify risk factors.

**Materials and Methods:** A total of 142 cervicals samples were collected from Senegalese women. The endocervix samples were used for identifying 14 types of HPV-HR of which (16 and 18 specified by their genotype) and (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 not specified) using Abbott m2000sp/m2000rt RealTime High Risk HPV.

**Results:** The prevalence of HPV-HR was 12.7%. The 12 types of not specified HPV-HR showed a prevalence of 10.6%. Types 16 and 18 were identified in a respective proportion of 1.4% and 0.7%, no co-infection was identified. The risk of developing HPV-HR infection is independently associated ($P > 0.05$) with the aspect of the cervix, age, ethnics groups, contraception, and the age first sexual intercourse. However, HPV-HR infection prevalence is significantly higher ($P<0.05$) in women that are single/widowed/divorced (41.7%), who went through multiples miscarriage (14.3%), with multiples sexual partner (16.4%), active smoking and alcohol consumption (40%).

**Conclusion:** These results highlight the need for a new preventive vaccine against all HPV-HR. The value of monitoring risk factors for impactful public health address is critical for the screening of HPV-HR in sub-Saharan women.

**Keywords:** Human papillomavirus; Prevalence; Genotyping; Abbott m2000rt/m2000rt; Senegal

## Introduction

Human papillomavirus and cervical cancer have become a major preoccupation for the medical and scientific international communities. Around 440 million people were exposed to HPV in 2012, of which 291 million were women [1]. Sub-Saharan Africa (24%), Eastern-Europe (21.4%) and Latin America are the most affected areas [2]. According to WHO cervical cancer will be responsible for 443,000 women's death per year with nearly 90% in Sub-Saharan Africa between now and 2030 [3]. Worldwide, HPV infections represent around 5.2% of human cancer [4]. Cancer proportion linked to HPV among women represents more than 10% off all cancer cases [5]. In comparison, 2.2% of cancers in developed countries and 7.7% in developing countries are linked to Human Papillomavirus High-Risk oncogene (HPV-HR) [6]. In United-States, the prevalence of the virus in women aged from 18 to 25 years was 14.3% for those who had one sexual partner; 22.3% for those who had two, and 31.5% for those who had three and above [7]. Women cervical cancer is attributed in 99% of all cases to a persistent infection of one or more HPV-HR genotypes [8].

Senegal is ranked 15th in the world in term of the incidence of cervical cancer [9]. In 2014, cervical cancer represented 30% of all deaths by cancer among women. Making it the most frequent death by cancer in Senegal, followed by breast cancer (15.5%) [10].

Up to now, 189 genotypes were identified which 120 are able to infect human [11]. The classification based on the oncogene power allowed to distinguish: Human Papillomavirus with High-Risk oncogenes (HPV-HR), generator of precancerous lesions, and the HPV low-risk oncogenes (HPV-LR) causing a benign lesion. Oncogene ability of the virus is driven by mucous tropism; however, some types induce cutaneous carcinoma [12]. HPV-HR are found in nearly all cervical cancer and in 25% of upper aerodigestive tract (UAT) cancer [13]. The 8 types of HPV most frequently found in cancers are: HPV 16, 18, 31, 33, 35, 45, 52 and 58 [14]. On a global scale, HPV 16 is associated with more than 50% and HPV18 to more than 15% of all cases of cervical cancer [15]. Around 36 to 40% of vulvar cancer and nearly 90% of vaginal cancer are linked to HPV [16]. Condylomas found in the anogenital mucosa are usually caused by HPV 6, 11, 42, 44, 50, 53 and 83 [17].

The continuous discovery and diversity of HPV-HR types emphasize the need for better screening techniques and preventive mechanism. With the aim to determine the presence of this virus

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in order to prevent its persistence activating the viral oncoproteins involved in the production of HPV-HR cancerous transformation of cervical cells. Development of molecular biology made it possible to establish their plurality, their tissue specificity, and their genotype-dependent pathogenicity.

The diagnosis of HPV infection is based on the detection of viral DNA by molecular hybridization techniques such as [18]:

1. Hybridization of nucleic acids whose hybridization *in situ* or dot-blot, having low sensitivity and requires a large amount of purified DNA.
2. Signal amplification tests such as hybrid capture 2 (hc2) having limitations in the individual identification of genotypes.
3. Nucleic acid amplification tests such as the Abbott Real-Time High-Risk HPV, a multiplex PCRs with good sensitivity with HPV genotyping abilities. Making it possible to specify the genotype (s) present in the samples and thus detecting co-infections as well [19].

The objective of our study is to increase knowledge of the prevalence, identify risk factors and generate data on HPV-HR types in Senegalese women using Abbott m2000sp/m2000rt with RealTime High-Risk HPV test kit. Specifically, to determine the overall prevalence of HPV-HR, genotypes 16 and 18, and other 12 unspecified HPV-HR, and the involvement of sociodemographic risk factors for HPV-HR.

## Material and Methods

### Type of study

Our prospective study was conducted at the Molecular Biology Laboratory of the Armed Forces AIDS Program at Ouakam Military Hospital, Dakar, Senegal from 2 August 2017 to 2 February 2018. Samples were collected as part of preventive screening for women and included a cervicovaginal smear and HPV screening to identify those at risk for developing cervical cancer. This prediction is based on HPV-HR genotype in cervical specimens.

### Study population

The study included 142 Senegalese women participating in the feasibility of HPV-HR genotyping test. Each participant completed a questionnaire on socio-demographic parameters such as age, marital status, history of miscarriage, age of first sexual intercourse, number of sexual partners, hormonal contraception usage, alcohol consumption, and active smoking. Information on color and abnormalities of the cervix was collected during the exam.

### Collection of cervical specimens

The 142 endo-cervical specimens were collected at Nabil Choucair Hospital (Dakar) where Pap smears were performed. The method involves introducing the speculum into the vaginal opening in a painless manner thereby exposing the cervix. With a cytobrush, endocervical cells were collected by swabbing in 180° rotation manner. The cytobrush is then introduced into a transport medium Abbott Multi-collect Specimen Collection Kit (Abbott GmbH & Co., Germany) containing 1.2 ml transport buffer, the sample was stored at -80° C until tested.

### HPV-HR molecular detection and genotyping method

The Abbott mSample Preparation System DNA and Abbott RealTime High Risk HPV Amplification Reagent Kit reagents (Abbott GmbH & Co., Germany and distributed by Abbott Molecular Inc., IL, USA) were used according to manufacturer instructions in the Abbott m2000sp/m2000rt for DNA extraction, amplification, and detection of HPV-HR genotypes.

Abbott RealTime High-Risk HPV Assay is a qualitative *in vitro* multiplex PCR test that targets HPV capsid L1 gene sequence in cervical samples [20]. This test contains an internal control (human β globin) and fluorescent probes allowing the detection of DNA separated from 14 HPV-HR: HPV16, HPV18, a pool of 12 HPV-HR (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and internal control. Each series has two controls, one negative and one positive integrated. The analyzer automatically validates the series and determines the presence or absence of HPV DNA and genotype.

### Data analysis

Four infection groups were defined for the analyzes: all the 14 HPV-HR, HPV 16, HPV 18 and the pool of 12 other HPV-HR (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The questionnaires were processed with Epi Info software version 7.2.2.6 (2018). The data was entered with the Microsoft Office Excel 2010 software. The charts and tables were built with the Microsoft Office Word and Excel 2010 software. With the R Studio software [R, version 1.1.423 (2011)], we have evaluated the association between the different studied parameters and the HPV-HR infection through the Pearson chi-square test which consists in determining the correlation coefficient P. Results were considered significant when $P<0, 05$.

## Results

### Prevalence of HPV-HR in the general population

Our results show that 18 out of 142 women had a positive result for HP-HR genotypes showing a prevalence of 12.7% (95% CI: 7.2-18.2). Genotyping revealed a prevalence of 1.4% and 0.7% respectively for HPV16 and HPV18. The other HPV-HR none 16 and 18 (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) had a prevalence of 10.6%. There was no co-infection, ie each infected woman harbored a single HPV-HR genotype (Table 1).

### HPV-HR prevalence according to the color of the cervix

We evaluated women's HPV-HR carriage according to the color of their cervix. In pink-colored (Normal) cervix (N=130), the prevalence of HPV16, HPV18 and other-HPV-HR were 1.5%; 0.8% and 8.5% respectively. For women with a red cervix (inflammatory) (N=12), our results showed the exclusive presence of other-HPV-HR at a prevalence of 33.3% (Table 1). There is no significant difference in HPV prevalence between red and pink cervix women ($P$=0.23).

### Prevalence of HPV-HR according to the cervix aspect

The cervix is abnormal if there is leucorrhea, metrorrhagia or polyps. HPV-HR prevalence in women with a normal cervix was 12.8%. They carry HPV16 and HPV18 in respective proportions of 1.6% and 0.8%. The prevalence of other-HPV-HR in women with cervical abnormalities was 11.8% (Table 1). The difference in HPV-HR prevalence between women with normal and abnormal cervix is not significant ($P$=0.58).

### HPV-HR prevalence by age (years)

In our study, women were classified into two age groups 30-40

years (N=85) and (41-55) years (N=57). The median age of the patients was 39 years ranging from 30 to 55 years. The prevalence of HPV-HR infection was 15.3% and 8.8% respectively in women aged 30-40 years and those between 41-55 years old. HPV16 was present exclusively in women of 40-55 years old and HPV18 in those of 30-40 years old with respective prevalence's of 3.5% and 1.2% (Table 1). There is no significant age difference in HPV-HR infection rates ($P$=0.61).

## Prevalence of HPV-HR according to marital status

We evaluated the carriage of HPV according to the marital status of the patient. The HPV-HR prevalence was 41.1% and 6.8% for single/divorced and married women respectively. HPV16 and HPV18 had an identical prevalence of 4.2% among single/divorced/widowed (Table 1). HPV-HR prevalence is significantly higher in single/divorced women than in married women ($P$=0.000067).

## Prevalence of HPV-HR according to contraception

The evaluation of HPV-HR carriage according to the presence or absence of a hormonal/no hormonal contraceptive showed an HPV-HR prevalence of 20.4% and 9.2% respectively in women not using contraception and in those using contraception. HPV16 was present exclusively in women using contraception and HPV18 only in those not using contraception with prevalences of 2% and 2.2% respectively (Table 1). The presence or absence of contraception in women does not affect the rate of HPV infection ($P$=0.36).

## HPV-HR prevalence according to the number of miscarriages

We evaluated the prevalence of HPV-HR in two groups of women with and without antecedents. It was noted that the HPV-HR prevalence is significantly higher among women who had at least 1 miscarriage (≥ 1), 14.3% compared to those who never had a history of miscarriage, 10.8% ($P$=0.034). HPV16 was detected only in women with a miscarriage at 2.6% and HPV18 only in women without miscarriage at 1.5%. Other HPV-HR are dominant in both group, 9.2% and 11.7% respectively in women without a history of miscarriage (Table 2).

## Prevalence of HPV-HR by age of first sexual intercourse

Questionnaire results showed that 82 (57.7%) of women had their first sexual intercourse at an early age (13 to 20 years) and 60 (42.2%) after 20 years. The average age of first sexual intercourse is 22 years ± 4.8 years ranging from 13 to 43 years of age. The prevalence of HPV-HR is 13.4% among women who had their first sexual intercourse between 13-20 years and 11.7% among women who had their first sexual intercourse after 20 years ($P$=0.37). HPV16 was detected with a prevalence of 1.2% and 1.7% among women who had their first relationship between (13-20) years and after 20 years respectively. HPV 18 was detected only in women who had their first relationship between years (Table 2) [13-20].

## Prevalence of HPV-HR by number of sexual partners

Multi-partnership is a contributing factor in the transmission of sexually transmitted infections (STIs). The average number of partners is 1.3 ± 0.4 ranging from 1 to 5 sexual partner(s). Women were divided into two groups, women who had only one sexual partner 75 (52.8%) and 67 women (47.2%) who had multiple sexual partners (≥ 2). The evaluation of HPV-HR carriage by partner number showed an HPV-HR prevalence was significantly higher at 16.4% among women who had multiple sexual partners than among women with only one partner 9.3% ($P$=0.000053). HPV16 and HPV18 were detected only in women with multiple sexual partners respectively in proportions of 2.9% and 1.5% (Table 2).

## HPV-HR prevalence by active smoking and alcohol

To evaluate the impact of active smoking and alcohol on HPV-HR carriage, women were split into two groups; 1, no alcohol usage nor smoking and 2, using alcohol or smoking tobacco. The prevalence of HPV-HR is significantly higher in group 2, 40% compared with 11.7% for group 1 ($P$=0.0062). In alcohol-smokers, only HPV18 and other-HPV-HR were present at an identical prevalence of 20%. Group 1 showed prevalences of 1.4% and 10.2% respectively for HPV16 and other-HPV-HR (Table 2).

| Parameters | N | HPV-HR Pos | HPV 16 | HPV 18 | Others HPV | *P*-value |
|---|---|---|---|---|---|---|
| | | N (%) [IC 95%] | N (%) [IC 95%] | N (%) [IC 95%] | N (%) [IC 95%] | |
| Global population | 142 | 18 (12,7); [ 7,2-18,2] | 2 (1,4); [ 0-3,3] | 1 (0,7); [ 0-2,1] | 15 (10,6); [ 5,5-15,7] | |
| **Color of the cervix** | | | | | | |
| Rose | 130 | 14 (10,8); [ 5,7-16] | 2 (1,5); [0-3,5] | 1 (0,8); [0-2,3] | 11 (8,5); [4-13,1] | 0.23 |
| Red | 12 | 4 (33,3); [25,5-41,1] | 0 (0); [0-2,1] | 0 (0); [0-2,1] | 4 (33,3); [25,5-41,1] | |
| **Nature of the cervix** | | | | | | |
| Normal | 125 | 16 (12,8); [7,3-18,3] | 2 (1,6); [0-3,6] | 1 (0,8); [0-2,3] | 13 (10,4); [5,4-15,4] | 0,58 |
| Abnormal | 17 | 2 (11,8); [6,5-17,1] | 0 (0); [0-2,1] | 0 (0); [0-2,1] | 2 (11,8); [6,5-17,1] | |
| **Age** | | | | | | |
| [30-40] | 85 | 13 (15,3); [9,4-21,2] | 0 (0); [0-2,1] | 1 (1,2); [0-3] | 14 (16,5); [10,4-22,6] | 0,61 |
| [40-55] | 57 | 5 (8,8); [4,1-13,5] | 2 (3,5); [0,5-6,5] | 0 (0); [0-2,1] | 3 (5,3); [1,6-9] | |
| **Marital status** | | | | | | |
| Married | 118 | 8 (6,8); [2,7-11] | 1 (0,4); [0-1,4] | 0 (0); [0-2,1] | 7 (5,9); [2-9,8] | 0,000067 |
| Single/Divorced/Widowed | 24 | 10 (41,1); [33-49,2] | 1 (4,2); [0,9-7,5] | 1 (4,2); [0,9-7,5] | 8 (33,3); [25,5-41,1] | |
| **Contraception** | | | | | | |
| None | 44 | 9 (20,4); [13,8-27] | 0 (0); [0-2,1] | 1 (2,2); [0-4,6] | 8 (18,1); [11,8-24,4] | 0,36 |
| Hormonal/None hormonale | 98 | 9 (9,2); [4,4-14] | 2 (2,0); [0-4,3] | 0 (0); [0-2,1] | 7 (7,1); [2,9-11,3] | |

HR: High Risk
Others HPV (It's mean others HPV High-Risk excepted HPV 16 and 18): 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68
HPV-HR: HPV 16, HPV 18 and others HPV
Normal cervix: Without anomalies
Abnormal cervix: Leucorrhoea, metrorragia or presence of polyps

**Table 1:** HPV-HR prevalence according to nature and color of the cervix, ages, marital status and contraception.

| Parameters | N | HPV-HR Pos | HPV 16 | HPV 18 | Autres HPV | *P*-value |
|---|---|---|---|---|---|---|
| | | N (%) [IC 95%] | **N (%) [IC 95%]** | **N (%) [IC 95%]** | **N (%) [IC 95%]** | |
| **Number of miscarriages** | | | | | | |
| 0 | 65 | 7 (10,8); [5,7-15,9] | 0 (0); [0-2,1] | 1 (1,5); [0-3,5] | 6 (9,2); [4,4-14] | 0,034 |
| ≥1 | 77 | 11(14,3); [8,5-20,1] | 2 (2,6); [0-5,2] | 0 (0); [0-2,1] | 9 (11,7); [6,4-17] | |
| **Age of the first sexual intercourse** | | | | | | |
| [13-20] | 82 | 11 (13,4); [7,8-19] | 1 (1,2); [0-3] | 1 (1,2); [0-3] | 9 (11); [5,9-16,1] | 0,37 |
| >20 | 60 | 7 (11,7); [6,4-17] | 1 (1,7); [0-3,8] | 0 (0); [0-2,1] | 6 (10); [5-14,9] | |
| **Number of sexual partners** | | | | | | |
| 1 | 75 | 7 (9,3); [4,5-14,1] | 0 (0); [0-2,1] | 0 (0); [0-2,1] | 7 (9,3); [4,5-14,1] | 5.00E-05 |
| >1 | 67 | 11 (16,4); [10,3-22,5] | 2 (2,9); [0,1-5,7] | 1 (1,5); [0-3,5] | 8 (11,9); [6,6-17,2] | |
| **Active smoking and alcohol consumption** | | | | | | |
| Alcohol/Tabac | 5 | 2 (40); [31,9-48,1] | 0 (0); [0-2,1] | 1 (20); [13,4-26,6] | 1 (20); [13,4-26,6] | 0,0062 |
| No alcohol/tobacco usage | 137 | 16 (11,7); [6,4-17] | 2 (1,4); [0-3,3] | 0 (0); [0-2,1] | 14 (10,2); [5,2-15,2] | |
| **Ethnic group** | | | | | | |
| Eastern ethnic group | 33 | 4 (12,1); [6,7-17,5] | 1 (3,0); [0,2-5,8] | 0 (0); [0-2,1] | 3 (9,1); [4,4-13,8] | 0,73 |
| Western ethnic group | 69 | 8 (11,6); [6,3-16,9] | 0 (0); [0-2,1] | 0 (0); [0-2,1] | 8 (11,6); [6,3-16,9] | |
| Southern ethnic group | 40 | 6 (15); [9,1-20,9] | 1 (2,5); [0-5] | 1 (2,5); [0-5] | 4 (10); [5-14,9] | |

HR: High-Risk
Autres HPV (It's mean others HPV high-Risk excepted HPV 16 and 18): 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68
HPV-HR: HPV 16, HPV 18 and others HPV
Estern ethnics: Pulhars people
Westhern ethnics: Wolofs
Southern ethnies: Serer, Jola, Ndiago, Soninke, Balanta and Susu

**Table 2:** HPV-HR prevalence according number of abortions, age of the first sexual intercourse, ethnic group, smoking and alcohol.

### Prevalence of HPV-HR infections by ethnicity

The relationship between the geographical area, the ethnic group and carriage of HPV-HR was studied. Ethnic association is grouped into three groups: Ethnies of the East: Fula people; Ethnies of the West: Wolofs; Ethnies of the South: Serer, Jola, Ndiago, Soninke, Balanta, Susu. The HPV-HR prevalence is 15%, 12.1%, 11.6% respectively among women of the South, East and West ethnic groups.

HPV16 was detected in the Eastern and Southern ethnic groups with respective prevalences of 3% and 2.5%. HPV18 is present only in Southern ethnic groups at 2.5%. Other HPV-HR are dominant with prevalences of 9.1%, 11.6%, and 10% respectively in the ethnic groups of East, West, and South (Table 2). The difference in HPV-HR carrying rates was not significant by ethnicity ($P = 0.73$).

## Discussion

Our study, although preliminary, provides important information on the molecular epidemiology of HPV-HR genotypes in women in Senegal. The molecular method (Abbott m2000sp/m2000rt) identifies specifically 2 types (HPV16 and HPV18) and a pool of 12 other high-risk genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) described here as other-HPV-HR (Not 16 not 18 HPV-HR).

The prevalence HPV-HR in our general study population is 12.7%. This rate is similar to the 12% reported in Mali, 12.5% in Congo, higher than 7.8% reported in Tunisia and lower than 25.4% reported in Bobo-Dioulasso, Burkina Faso [21-24]. In Senegal, an HPV-HR prevalence of 17.4% was described in the Dakar region [25]. However, data on the distribution of HPV according to risk factors are not well documented in Senegal. It is recognized that the distribution of HPV genotypes differs geographically and may influence the efficacy of vaccines that primarily target genotypes 16 and 18 [26]. Southern Africa (57.3%), East Africa (42.2%), West Africa (27.8%), and East Asia (China, 57.7%) reported higher prevalence compared to North Africa (12.8%), Latin America (16.1%) and Eastern Europe (21.4%) [2,27,28]. The difference in diagnosis and access to care may explain regional disparities in women's level of infection. Vinidhini et al. show an average HPV prevalence of 42.2% and 22.6% respectively for resource-limited and developed countries [28].

By looking at the genotypes detected among the 14 HPV-HRs according to our molecular detecting method, our results showed low prevalences of HPV16 (1.4%) and HPV18 (0.7%) and a predominance of other-HPV-HR with a prevalence of 10.6% in the overall study population. A higher prevalence (43%) of HPV-HR type '30s and '50s was described with HPV genotype 35, 31, 52 and 58 more commonly found among women in Ouagadougou, Burkina Faso [24]. Another study showed that HPV39 (18.5%), HPV52 (16.7%), HPV18 (14.8%) and HPV35 (13%) were the most common genotypes among women in Bobo-Diolasso, Burkina Faso [29]. In the Fiji Islands, HPV-HR had a prevalence of 13.6% in women with HPV16, 52, 56, 59 as the dominant genotypes [30]. These genotypes mentioned above are found in our pool of 12 non-vaccinal genotypes with a high prevalence in our population even if the identification of genotypes is not individualized by our method. Indeed, there is a predominance of none 16 and none 18 genotypes HPV-HR in most countries of sub-Saharan Africa, which differs from the distribution in Europe, North America where a predominance of genotypes 16 and 18 have been described [28,31]. This distribution of HPV-HR genotypes results in the establishment of other preventive vaccines that take into account HPV-HR none 16 and none 18.

Our results showed that the prevalence of HPV-HR was higher in women with a red (inflammatory) cervix, 33.3% compared to 10.8% in pink-collar women ($P$=0.23). In women who had a normal cervix, HPV-HR carriage was 12.8% versus 11.8% ($P$=0.58) for those with cervical abnormalities such as leukorrhea, polyps, and metrorrhagia. Thus, the positivity of HPV-HR infection is independently associated with the color of the cervix or the presence of leucorrhea, metrorrhagia or polyps. This finding was also reported in Tunisia, where 62.5% of women infected with HPV-HR had a normal cervix [23].

Regarding the involvement of socio-demographic parameters such as age, women between 30-40 years old are more exposed to HPV-HR infection with a prevalence of 15.3% against 8.8% among women between (41-55) years ($P$=0.61). The trend in our results is consistent with that reported in the Fijian Islands with an HPV prevalence of 25.8%, in women between 25-34 years old and 18.6% among those aged 55-64 years ($P$<0.001) [30]. Indeed, the majority of HPV infections are transient and its decrease with age results from the effectiveness of immune defenses [31,32]. Bennani et al. showed the opposite, the age group>45 years was the most affected among women in Morocco [33]. HPV16 and HPV18 are more prevalent in women over 30 compared to those under 30 in Burkina Faso, which was observed for HPV18 in our study [24].

The prevalence of HPV16, HPV18, and other-HPV-HR is significantly higher among single/divorced/ widowed women than among married women ($P$=0.000067). This significant difference supports the hypothesis that unmarried, widowed or divorced status influences the risk of HPV-HR infection. Our results are consistent with those reported in Mali, Fijian Islands and Burkina Faso, which show that single women (single/divorced/ widowed) have higher HPV prevalence than married women [21,29,30]. In Iran HPV were present at high levels among divorced women but also among the married in a polygamous regime and/or husbands were absent more than 7 days per month [34]. In Cameroon, the opposite has been described with higher prevalences among brides (53.9%), followed by widows (34.5%) and singles ladies (11.5%) [35].

Our results showed that women using contraception (hormonal / non-hormonal) are less likely to carry HPV-HR, 9.2% than women without contraception 20.4%. Other studies showed similar results [29,30]. However, our study did not identify a link between contraception usage and their level of HPV-HR infection ($P$=0.36). Another study conducted in Italy also found a lack of relation between HPR-HR carriage and the use of contraceptive method by women [36]. However, in Thailand, it has been shown that HPV infections persist more in women on contraception, which would increase HPV prevalence in this population by default of clearance independently of sexual behavior [37]. Raising the hypothesis of the impact of the contraceptive pill as a risk factor is variable for the carriage of HPV-HR. Some data suggest that the risk factor associated with hormone intake, such as contraceptive, is linked to cervical cancer [38].

HPV-HR prevalence is significantly higher among women who had a history of miscarriage (≥ 1) compared to those with no miscarriage history ($P$=0.034). Our study showed a connection between abortion and HPV-HR infections. Another study in Morocco also reported higher prevalence among women who had at least 1 miscarriage compared to women who never experienced one [39]. Multiple abortions weaken the lining of the cervix, making the epithelium more vulnerable to infections. Women with a history of miscarriage have a 2-fold increased risk of developing cervical cancer and those who have suffered two or more face a risk 5 times higher [40].

The HPV-HR prevalence according to the age of the first sexual intercourse was 13.4% among women who had their first sexual intercourse between 13-20 years old and 11.7% among women who had their first sexual intercourse after 20 years of age ($P$=0.37). This higher HPV-HR prevalence among women with early sexuality has been demonstrated in Morocco [39]. According to our data, the age of first sexual intercourse cannot be significantly considered as a risk factor associated with carriage of HPV-HR genotypes. The same observation has been made in other studies around the world however, some studies came to different conclusions [29,31]. Some data supports that the risk factor associated with early sexual intercourse is cervical cancer, rather than HPV infection [41].

The prevalence of HPV-HR infection was significantly higher among women with multiple sexual partners (≥ 2) than among those with a single sexual partner ($P$=0.000053). Our results show a correlation between the number of sexual partners and their level of HPV-HR infection. Similar findings were described in the United States [42] and in Ouagadougou, Burkina Faso [31]. One study in sub-Saharan women did not report a link between the number of sexual partners and HPV-HR carriage [22]. Some authors challenge the idea that current sexual behavior is not the only risk factor but rather persistence and latency of HPV reactivating in older women [43].

Our results showed that alcohol users and tobacco smokers are significantly more likely to carry HPV-HR compared to those who do not use alcohol or tobacco ($P$=0.0062). Our results are in agreement with those of Vinodhini et al. [28]. While active smoking is a risk factor for cervical cancer, it was also shown that passive smoking increases the persistence of HPV-HR and doubles the risk of this cancer [44].

As far as ethnicity is concerned, the ethnic groups of the South (Serer, Jola, Ndiago, Soninke, Balanta, Susu) are more affected than those of the East (Fula People) and the West (Wolofs) ($P$=0.73). Ethnicity does not appear to influence significantly the prevalence and distribution of HPV-HR, all ethnicities may harbor high-risk oncogenic genotypes in the same proportions. Host genetics may influence the clearance or persistence of HPV. In fact, the genetic polymorphism of the host immune system, in particular, the HLA polymorphism, influences the immune response and the viral persistence are involved in the occurrence of tumors related to the viral type [45].

In perspective, this study was conducted a small population increase the number of patients would promote stronger statistical evidence, allowing a better knowledge of risk factors in Senegal and Sub-Saharan Africa. Also, specific genotyping for viruses screen would be interesting to better understand the distribution of HPV-HR serotype in the region. These studies would be necessary to guide prevention programs against HPV and cervical cancer. Increasing risk factor knowledge would permit more targeted public health approach, ideally promoting voluntary HPV-HR testing in relation to cervical-vaginal smear for at risk population.

If types 16 and 18 were present for which a vaccine is available, there remain other high-risk oncogenic genotypes that are not affected by the vaccine and that predominate in our series. This diverse genotype distribution raises the need to develop new vaccines taking into account other types of circulating HPV-HR.

## Conclusion

Our results showed a lower prevalence of HPV-HR 16 and 18 and higher for other HPV-HR genotypes (31,33, 35,39,45,51,52,56,58,59,66,68) in Senegalese women. Single/widowed/ divorced status, history of miscarriage, multiple sexual partners, active smoking, and alcohol are risk factors for HPV infection. Age, ethnicity, contraception, and age of first intercourse are not associated with the risk of HPV infection. These results contribute to a better understanding of the distribution of HPV and their risk factor in Sub-Saharan Africa.

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