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Original Article

Human papillomavirus among women living with Human Immunodeficiency Virus in Morocco A prospective cross-sectional study

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Abstract

Introduction: Women infected with human immunodeficiency virus (HIV) have a higher risk of contracting human papillomavirus (HPV) infections and are more prone to develop cervical cancer. The objective of this study was to determine the prevalence of HPV and its association with risk factors among Moroccan women living with HIV/AIDS.

Methodology: We enrolled 251 HIV-infected non-pregnant women in Morocco from February 2013 to September 2016. Sociodemographic, lifestyles, behavioral and clinical data were collected. Polymerase chain reaction followed by sequencing were performed for molecular detection and HPV genotyping in cervical samples, respectively.

Results: Abnormal cervical smears were found in 34/246 patients (13.82%). The overall prevalence of HPV was 74.50%. HPV 58 was the most prevalent (39.29%) followed by HPV 18 (10.71%), HPV 70 (8.93%), HPV 33 (7.14%), HPV 6 (6.25%) and other genotypes (< 3%). Overall, high-risk HPV (HR-HPV) types were present in 75% (84/112) of patients and the prevalence of HR-HPV types in samples with abnormal Pap was higher than in normal Pap (55/83, 66.27% vs. 28/83, 33.33%, $p < 0.0001$). Univariate analyses showed that none of the socio-demographic and behaviors factors was associated with HPV infection. Moreover, Pap results were not affected by HPV status ($p = 0.532$). Whereas, CD4 T-cell counts above 200/mm^3 at enrolment were apparently not protective to HPV infection. We found a high prevalence of HPV infection and HR-HPV types among HIV-positive women that significantly associated with abnormal Pap.

Conclusion: Our findings suggest a high prevalence of HPV infection with high-risk types was observed among HIV-positive women warrant to implement a regular screening by Pap smear.

Key words: AIDS; cervical cytology; human immunodeficiency virus; human papillomavirus; prevalence.

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Introduction

According to the GLOBOCAN 2012, cervical cancer (CC) is the second most common cancer among Moroccan women after breast cancer (ASR: 14.3 per 100,000) with approximately 2258 new cases occurring in 2012 [1]. Human papillomavirus (HPV) is the main etiological agent for cervical lesions worldwide and the persistence of high-risk HPV infection is closely associated with the development of intraepithelial neoplasia and cervical cancer [2]. The vast majority of cervical HPV infections resolve or become latent and undetectable, however in a subset of women, the infection persists [3,4]. This heterogenous virus family includes more than 200 genotypes [5]. Previous studies in Moroccan women have been reported an inconsistent prevalence of HPV with 15.7% [6] in Rabat and 42.5-43.1% in Fez region [7,8]. However, the prevalence was much higher, ranging from 62% to 92%, among Moroccan women with CC [9-12]. The joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that 30,000 (22,000 – 40,000) Moroccans were living with HIV/AIDS at 2012, and women aged 15 years old and older living with HIV was 11,000 (8,500 – 14,000). In addition, the number of deaths was estimated to 1,200 (< 1,000 – 1,800) [13]. Previous studies suggested that women infected with HIV have a higher prevalence of HPV infection [14,15]. In fact, the HIV/acquired immunodeficiency syndrome (AIDS)-

related immunosuppression increases the risk of persistent HPV infection resulting in higher incidence and prevalence of cervical intraepithelial neoplasia (CIN) lesions and a more prone to progress to cancerous lesions of the cervix and seems to occur at a younger age [15-17]. Moreover, highly active antiretroviral therapy (HAART) has increased the life-spans of HIV-infected women, thus has increased their risk for a prolonged period of persistence of HPV infection [18]. In addition, the impact of HAART on HPV-associated lesions remains controversial [19,20]. Furthermore, previous data reported that HIV-positive women have a 2- to 12-fold higher risk of CIN lesions compared to HIV-negative women [21,22]. Therefore, the accurate assessment of the burden of HIV/HPV co-infection is of great importance. Data on HPV infection among HIV-infected women in Morocco are scarce. This study evaluated the prevalence of HPV infection among a large cohort of HIV-infected women, never screened for HPV. Pap smear and factors associated with prevalence of HPV were explored and the distribution of HPV types in a subsample of the women was assessed.

Methodology

Study population

A longitudinal cohort study has been conducted from February 2013 to September 2016. All women coming to the Infectious Disease Center, University Hospital Center, Ibn Rochd in Casablanca for routine antiretroviral therapy or pre-ART care were and given the opportunity to participate in the study. Women were excluded if they were pregnant, previous hysterectomy, having prior cryotherapy or cervical cancer. After giving written informed consent, every participant was interviewed and completed a structured questionnaire on sociodemographic, lifestyles, behavioral and clinical data. The Ethics Committee of Casablanca approved the study in 2012, and 251 HIV-infected non-pregnant women from different geographic parts of Morocco were enrolled.

Clinical laboratory data

$CD4^+$ T-cell counts were enumerated by flow cytometry on a three-color FACSCallibur flow cytometer (Becton Dickinson Immunocytometer System, San Jose, USA). Automated extraction, amplification, and quantification were performed with the Cobas Ampliprep/Cobas TaqMan 48 analyzer system version 2.0 for HIV RNA viral load (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) following the Roche manufacturer's standard guidelines.

Cytologic analysis

Cervicovaginal smears samples were collected by physicians using a Cytobrush. Cervical swabs are placed in specimen transport medium (DNAPAP Cervical Sampler, Qiagen, Germantown, USA) for cytology and HPV detection. The Papanicolaou smear on the slide in a monolayer was performed for cytological study. Smear abnormalities were classified using the Bethesda system into five ordered categories (normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) or high-grade intraepithelial lesion (HSIL) and invasion cancer).

The remaining samples were stored in transport Cellsolutions General Cytology Preservative medium (Cell Sollutions, Greensboro, USA) at -20 °C until use.

DNA isolation and HPV testing

DNA was isolated from the cervical swab specimens using QIAamp blood DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions in a biosafety bench and eluted in 200 µL of sterile water. The DNA samples were stored at -20 °C until use. The samples were screened for the presence of HPV using nested PCR method consisting of the MY09 (5'-CGTCCMARRGAWACTGATC-3')/MY11 primer (5'-GCMCAGGWCATAAYAATGG-3') as outer primers for the first round and inner primers GP5+ (5'-TTTGTTACTGTGGTAGATACTAC-3') and GP6+ (5'-GAAAAATAAACTGTAAATCATATTC-3') and β-globin as described previously [23,24]. The first-round PCR was performed for 45 cycles (94 °C for 20 s, 55 °C for 20 seconds and 72 °C for 1 minute) and a final extension step at 72 °C for 7 minutes in a 25 µL reaction volume containing 50 ng of extracted DNA, 1X PCR buffer, 200 mM of each dNTPs, 1.5 mM $MgCl_2$, 20 pmol/µL of each outer primer and 2.5U Taq DNA polymerase (Invitrogen, Paris, France). PCR product from the first-round was subjected for the second-round for 45 cycles (94 °C for 30 s, 43 °C for 1 minute and 72 °C for 1 minute) and a final extension step at 72 °C for 7 minutes in a 25 µL reaction volume under the same conditions. Negative and positive controls were included in each assay. After the second round, a 145 bp fragment was obtained and detected by electrophoresis on a 2% agarose gel.

HPV typing

Positive PCR products were purified using the Exonuclease I/Shrimp Alkaline Phosphatase

(Amersham, GE Healthcare, Hatfield, UK) and sequenced using BigDye Terminator version 3.1 kits and an ABI PRISM 3130 DNA automated sequencer (Applied Biosystems, Foster City, CA, USA) with GP6+ primer. The sequences were edited using BioEdit software and were subjected to analysis using BLASTN (https://blast.ncbi.nlm.nih.gov/) and Papillomavirus Episteme (PaVE) (https://pave.niaid.nih.gov/#analyze/l1taxonomytool) to identify the HPV type [25].

Statistical analysis

Sample size was performed as described previously [26]. Quantitative variables are presented using the median (interquartile range [IQR]) and median with range as appropriate. Qualitative variables were described using percentages and their 95% confidence interval (CI). For categorical variables, the Chi-square test was used. Age was dichotomized into > 30 years and ≤ 30 years; this categorization was used to reflect the WHO 2014 guideline concerning cervical screening. The Chi-square and Chi-square test for trend or the Fisher's exact test were used for evaluating associations between categorical variables. Multivariate analysis was used to estimate the odds ratio (OR) and their respective 95% CI. All p-values were two-sided and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad PRISM version 6.0e (GraphPad Software, San Diego, CA, USA).

Results

The study included 251 HIV-infected women who have not had a Pap smear before in their life. Socio-demographic, behavioral, reproductive and lifestyle characteristics of the cohort are summarized in Table 1. The median age was 39 years [Interquartile range (IQR): 32-47], with median age of first intercourse of 19 years (IQR: 17-21), the median age of first pregnancy was 23 years (IQR: 20-27) and the majority of women were married or divorced (69.05%) and used oral contraceptive (66.93%). The educational level was illiterate for 36.25%, primary school for 32.27%, secondary school for 24.70% and high school for 6.78% of the women, respectively. Of the 251 patients, 68.53% were unemployed. The median CD4 count was 532 cell/mm^3 (range: 5-1896). The median viral load was 1.6 Log_{10} copies/mL (range: 1.30-6.81). At enrollment, the majority of patients (98.80%) were under HAART (Table 1).

The overall prevalence of HPV was 74.50% (187/251) (95% CI, 69.11-79.89) of cases were positive

Table 1. Selected baseline of socio-demographic, reproductive and lifestyle characteristics of study population.

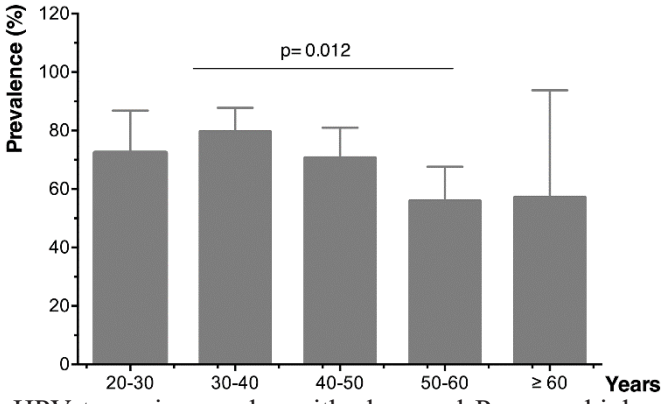
Characteristics	Women living with HIV (N = 251)
Median age (IQR), years	39 (32-47)
Marital status (N, %)	
Married	97 (38.49)
Single	36 (14.29)
Widowed	42 (16.67)
Divorced	77 (30.56)
Educational level (N, %)	
Illiterate	91 (36.25)
Primary school	81 (32.27)
Secondary school	62 (24.70)
High school	17 (6.78)
Employment (N, %)	
Yes	79 (31.47)
No	172 (68.53)
Smoking status (N, %)	
Never smoker	181 (72.11)
Smoker	70 (27.89)
Use of cannabis (N, %)	
Yes	15 (5.98)
No	236 (94.02)
Alcohol intake (N, %)	
Yes	70 (27.89)
No	181 (72.11)
Oral contraceptive use (N, %)	
Yes	168 (66.93)
No	83 (33.06)
Diabetes (N, %)	
Yes	94 (37.45)
No	157 (62.55)
Median age at first intercourse (IQR), years	19 (17-21)
Median age of first pregnancy (IQR), years	23 (20-27)
Number of pregnancy (N, %)	
None	37 (14.74)
1	61 (24.30)
2	72 (28.69)
3	33 (13.15)
4	26 (10.36)
More than 4	22 (8.70)
Multiple sex partners (N, %)	
Multiple	135 (53.78)
Single	116 (46.21)
Sex partner circumcised (N, %)	
Yes	231 (92.03)
No	20 (7.97)
Rape	
Yes	19 (7.57)
No	232 (92.43)
History sexually transmitted infection (N, %)	
Yes	148 (58.96)
No	103 (41.04)
Syphilis (N, %)	26 (10.36)
Cervicitis (N, %)	47 (18.73)
HBV/HIV coinfection (N, %)	24 (9.56)
HCV/HIV coinfection (N, %)	15 (5.98)
HAART (N, %)	248 (98.80)
Median CD4+ T count (cell/mm^3), (range)	523 (5-1896)
Median viral load (Log10 copies/mL), (range)	1.60 (1.30-6.81)
HIV stage (N, %)	
A	88 (35.06)
B	54 (21.51)
C	109 (43.34)
Condyloma (N, %)	8 (3.19)
Cancer* (N, %)	10 (3.98)

*Breast cancer, Kaposi sarcoma; IQR: Interquartile range; HAART: highly active antiretroviral therapy; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

for HPV DNA. Stratification according to age showed a significant difference between age group (p = 0.012) with a peak prevalence in women in the (30-40 years) age range (79.5%) followed by a decline and in the [50-60] age group, the prevalence (55.88%) was the lower (Figure 1). HPV typing was successful only in 112 among 187 (59.89%) patients and showed 27 different types: 6, 12, 13, 16, 18, 25, 31, 33, 35, 45, 52, 53, 54, 56, 58, 62, 66, 70, 81, 82, 89, 96, 130, 135, 172 and 178 (Figure 2). However, for the remaining samples (n = 75) we are unable to determine the HPV type by sequencing, suggesting that those samples were infected with more than one type of HPV (multiple infections). Moreover, HVP58 was the most common genotype (39.29%) followed by 18 (10.71%), 70 (8.93%), 33 (7.14%), 6 (6.25%) and other genotypes less than 3% (Figure 2). Overall, high-risk HPV (HR-HPV) (HPV 16, 18, 31, 33, 35, 42, 52, 58 and 70) types were present in 75% (84/112) of patients and low-risk (LR-HPV) (HPV 6, 13, 54, 56, 62 and 81) was found in 12.50% of cases (14/112) (Figure 2).

Abnormal cervical smears (ASCUS, LSIL or HSIL) were found in 34/246 patients (13.82%), the majority of which (7.72%) correspond to LSIL. Normal samples were found in 35.37% (87/246) of samples and non-displastic inflammation was observed in 50.81% (125/246) of cases. No invasive or *in situ* cervical cancer cases were observed (Figure 3). Pap results were considered abnormal if they were ASCUS or a more severe squamous lesion. Pap results at baseline according to HPV status and no significance between abnormal (80%) and normal Pap (73.11%) was observed (p = 0.532). However, the prevalence of HR-HPV types in samples with abnormal Pap was higher than in normal Pap (55/83, 66.27% vs. 28/83, 33.33%, p < 0.0001; OR = 3.85, 95% CI: 2.02–7.34).

Figure 1. Prevalence of human papillomavirus infection according to age group.



Data analysis showed that none of the demographic factors was associated with HPV status (Table 2). Whereas, CD4 T-cell counts above 200/mm^3 at enrolment were apparently not protective to HPV infection.

Discussion

HPV prevalence and genotype distribution varied in a different geographic area (15-21). Two prophylactic HPV vaccines, Gardasil (Merck & Co, White House Station, NJ) and Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) were licensed in Morocco since 2008. In addition, all women included in this study were not previously vaccinated against HPV. To our knowledge, this is the first large report on HPV prevalence among women living with HIV/AIDS in Morocco. In this study, we found a significantly higher

Figure 2. Prevalence of human papillomavirus types in women living with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome. LSIS: low-grade squamous intraepithelial lesion; ASCUS: atypical squamous cells of undetermined significance; HSIL. or high-grade intraepithelial lesion.

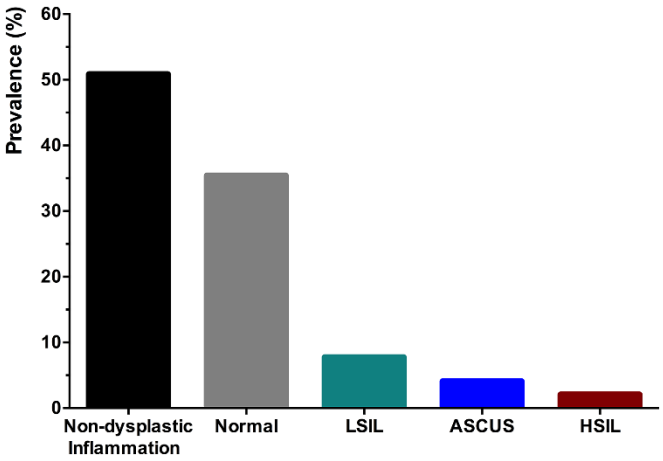


Figure 3. Cervical cytology in women living with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome.

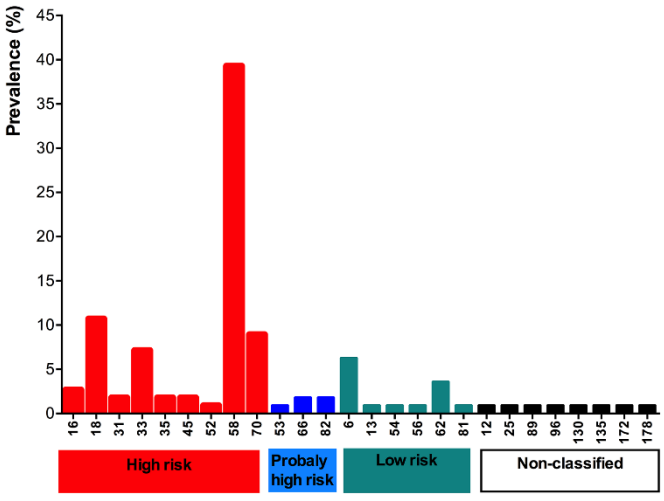


Table 2. Association between HPV infection and socio-demographic, behavioral, reproductive and lifestyle variables.

Characteristics	Total (N)*	HPV positive N (%)	Odds ratio (95% CI)	P-value
Age (years)				
< 30	36	26 (72.22)		
≥ 30	215	161 (74.88)	0.87 (0.39-1.92)	0.734
Educational level				
Illiteracy				
Illiterate	91	72 (79.12)	1.56 (0.77-3.14)	0.214
Primary school	81	59 (72.84)	1.10 (0.55-2.19)	0.783
Secondary /High school	79	56 (70.89)	1	
Employment				
Yes	79	60 (75.95)		
No	172	127 (73.84)	1.12 (0.60-2.07)	0.721
Smoking status				
Never smoker	181	130 (71.82)		
Smoker	70	57 (81.43)	1.77 (0.87-3.41)	0.117
Oral contraceptive use				
Yes	168	126 (75)	1.08 (0.59-1.97)	0.257
No	83	61 (73.49)		
Multiple sex partners				
Single	116	82 (70.68)		
Multiple	135	105 (77.78)	1.45 (0.82-2.56)	0.198
Number of pregnancy				
< 3	169	123 (72.78)		
≥ 3	82	64 (78.04)	1.33 (0.71-2.48)	0.369
Length of time sine HIV diagnosis (years)				
< 5	147	107 (72.79)		
≥ 5	104	80 (76.92)	0.80 (0.45-1.44)	0.459
CD4+ T count (cell/mm^3)				
< 200	31	18 (58.06)		
≥ 200	211	163 (77.25)	0.41 (0.19-0.89)	0.022
HIV viral load (Log10 copies/mL)				
< 4	89	61 (68.54)		
≥ 4	159	123 (77.35)	0.64 (0.35-1.14)	0.128
History sexually transmitted infection (N, %)				
Yes	148	107 (72.30)	0.75 (0.42-1.35)	0.337
No	103	80 (77.67)		

*Missing data.

prevalence of HPV infection in women living with HIV/AIDS (74.50%) and HR-HPV types were present in the vast majority of cases (75%). These findings highlighted that the HPV prevalence in the HIV-positive Moroccan women was significantly higher than observed in the general population (15.7%-43.1%) [6-8]. This seems to be in line with other previous reports which documents high proportion of HIV-infected women carrying varieties of HR-HPV types [27,28]. Furthermore, previous reports showed that HIV-infected women had a seven-fold increased rate of persistence of HR-HPV [29]. In addition, high HPV infection prevalence in HIV-positive women has been reported in Italy (44%) [30], US (54%-73%) [31], Brazil (48-78.8%) [32-35], South Africa (52.4%-74%) [36,37], Tanzania (54.1%) [27]and Burkina Faso (66.1%) [38]. However, a preliminary study carried out among 87 HIV-positive women in the Souss region (South of Morocco) reported a prevalence (39.3%) less than observed in our study [39].

The highest prevalence of HPV infection was observed among women in the [30-40] age group (79.5%). This data is in agreement with previous findings highlighted that HPV infection is more common among women younger than 34 years and decreased in older women supports that HPV is transmitted through sexual relations [40,41]. However, a previous study in uninfected-HIV women showed that age-specific HPV distribution presented with a first peak at younger ages (< 25 years) and a rebound at older ages (≥45 years) [42]. Furthermore, HPV prevalence peaked below age 25 or 35 years, and declined with age

in Italy, the Netherlands, Spain, Argentina, Korea and in Lampang, Thailand, and Ho Chi Minh, Vietnam was reported [43]. This was not the case in Songkla, Thailand nor Hanoi, Vietnam, where HPV prevalence was low in all age groups. In Chile, Colombia, and Mexico, a second peak of HPV prevalence was detected among older women [43].

Type-specific distribution revealed that HPV58 was the most prevalent type. This result seems to be in line with previous reports [36,44]. In contrast, this molecular epidemiology profile was not consistent with observed in HIV-negative women [6-8] or women with CC in Morocco [9-12], in whom HPV 16 and 18 predominate. Such discrepancies between groups (HIV-positive and HIV-negative) has been reported in previous studies and may partly due to immune system deficiency [30,45].

Current HPV vaccines include high-risk types 16 and 18 for inducing protective immunity. Interestingly, less than 12% of the HPV-infected women herein studied carried any of these two types. Moreover, a recent study conducted by the International Agency for Research in Cancer in HPV-infected women showed that HPV 58 was associated with invasive cervical cancer in eastern Asia [44]. These findings as well as ours, point to the need of reviewing strategies of vaccine development based on HPV molecular epidemiology.

In our study, 13.82% of HIV-infected women had Pap smear abnormalities. This data corroborate previous reports [46,47]. However, this rate is much lower than the rates reported in sub-Saharan Africa, which ranged from 49.8% to 73% [48] and India (27.5%) [15].

In this study, we found a high prevalence of HR-HPV types in women with abnormal Pap (66.27% vs. 33.33%, $p < 0.0001$) than normal Pap. This finding seems to be in line with previous data highlighting the potential carcinogenic effect of HR-HPV types [2,29,44]. Moreover, as we mentioned above that those women had never been screened by regular Pap or HPV test together with the high prevalence of cervical HR-HPV infections within, is a worrying situation and suggests the need for urgent screening and education of the wider population.

Data from previous studies found that cofactors increased the positivity of HPV among women living with HIV such as age greater than or equal to 35 years and greater partners [49,50]. However, in our study, none of the demographic factors was associated with HPV infection. In contrast, CD4 T-cell counts above 200/mm^3 at enrolment were apparently not protective to HPV infection. This data are in agreement with previous reports [50].

Conclusion

A high prevalence of HPV infections with high-risk types were observed among HIV-positive women in our investigation warrants to implement a regular screening by Pap smear. Moreover, this study demonstrated that HR-HPV infection was common among HIV-positive women with abnormal cytology findings. Furthermore, the inclusion of HPV58, 33, 70 in the next-generation HPV vaccines is of great importance and may improve the vaccine efficiency.

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