

Research Article

Prevalence, Genotype Distribution and Risk Factors of Human Papillomavirus in Moroccan Women

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Abstract

In Morocco, cervical cancer and precancerous lesions are still a public health problem and many efforts are made to improve women's awareness on Human papillomavirus (HPV) infection and methods preventing its dissemination. This study was planned to evaluate HPV prevalence and to assess the predominant HPV genotypes circulating in Morocco. A total of 360 women, attending caravans of sensitization and screening for cervical lesions were recruited from 9

different regions of Morocco. Socio-demographic, familial and medical data of participating women were recorded. HPV testing and genotyping were performed by Polymerase Chain Reaction and DNA direct sequencing. The overall HPV prevalence was 30.28% of the recruited women. Of the positive samples, 61.46% were infected with a high-risk HPV genotype, whereas 38.54% showed a low-risk HPV genotype. The most prevalent high-risk HPV genotypes were HPV 16, 59, and 18 with a prevalence of 25.68, 11.92 and 8.25%,

respectively. Association between HPV infection and some potential risk factors was also assessed and showed a significant association with age, living environment (urban vs. rural) and familial status ($p < 0.05$). In conclusion, the present study clearly showed that HPV infection among Moroccan women is high, with a predominance of HPV 16 and 18, suggesting that the two available vaccines in Morocco, Gardasil and Cervarix, could be of a great interest to protect women against HPV infection and limit HPV dissemination in the whole country.

Keywords: HPV infection; HPV genotypes; cervical cancer; Moroccan women

Introduction

Worldwide, cervical cancer (CC) is the fourth most common type of cancer among women, with 569,847 new cases diagnosed in year 2018 alone, and approximately 87.3% of these occur in low- and middle-income countries (LMIC), according to the incidence data from GLOBOCAN database (<https://gco.iarc.fr/today>). Currently, there is scientific evidence that Human papillomavirus (HPV) play a central role in CC development and HPV DNA is found in 95 to 100% of cases [1]. HPV infection of metaplastic epithelium at the cervical transformation zone, is considered as the first step of CC development that occurs after viral persistence and progression of persistently infected epithelium to cervical pre-cancer and cancer [2]. To date, more than 200 HPV genotypes have been identified [3], but the attention is still focused on 14 HPV types which are the most involved in pre-cancerous and cancerous lesions of the cervix, and frequently found in mucosal epithelium of the genital tract and other mucosal areas of the human body [4]. HPVs are classified according to their involvement in the genesis of benign or malignant lesions. Accordingly,

low-risk oncogenic HPVs (LR-HPV) are associated with anogenital benign warts, predominantly caused by HPV 6 and 11; and high-risk oncogenic HPVs (HR-HPV) are clearly associated with malignant cervical lesions including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 [2, 5–7]. Scientific data clearly showed that HPV 16 and 18 are the most frequently found genotypes in association with CC worldwide and are consequently considered as the most oncogenic types. However, prevalence of other genotypes is related to geographic or ethnic distribution [8].

In Morocco, CC is considered as a public health problem and data from two distinct cancer registries show that this malignancy ranks as the second most common cancer among women. CC incidence in Morocco is the highest in the North African region with an age-standardized incidence rate (ASR) of 15 per 100 000 women in Casablanca [9] and 13 per 100 000 women in Rabat [10]. In fact, an increasing East-West gradient is observed in the North African region, ranging from as low as 2.3/ 100,000/ year women in Egypt to as high as 17.2/100,000 women in Morocco (<https://gco.iarc.fr/today>).

Evaluation of HPV distribution in CC cases was performed on patients recruited in cancer's hospitals coming from different regions of Morocco showed a predominance of HPV 16 and 18 [11–13]. Moreover, HPV16 and 18-based prophylactic vaccines preventing persistent viral infections and HPV associated cervical lesions were introduced in Morocco, and are in increasing use. There is however evidence that the success of this prophylactic approach is depending on the HPV genotypes circulating in Morocco. Unfortunately, we lack data of the overall HPV prevalence among women with normal and abnormal cytologies across all the Moroccan territory, and to the

best of our knowledge, only two published studies have been conducted and have limited women recruitments in Rabat [14] and Fez region [15].

Therefore, this study was planned to evaluate the epidemiologic characteristics of HPV genotypes circulating in different regions of Morocco and to evaluate the associated risk factors in groups of women with no cervical symptoms, for better management of HPV detection, prevention and CC management in Morocco.

Materials and methods

Study design

The study was conducted in the frame of a caravan of sensitization and screening for cervical lesions concerning nine regions of Morocco from March 2009 to April 2014. A total of 360 women attending the medical caravans for simple gynecological control were recruited: Casablanca (98 women), Azemmour (23 women), Khmis Zmamra (36 women), Marrakech (30 women), Berkane (27 women), Beni Mellal (49 women), Haouz (26 women), Goulmim (46 women) and Zawyat Ahansal (25 women).

At the time of their visit, a face-to-face interview was conducted with each woman to complete a personal and socio-economic questionnaire in order to collect specific information on age, habitat, age of menarche, marital status, number of children, age at first birth, history of sexually transmitted diseases and smoking habits. For each participant, cervical swab was collected using Cervex brush. Cells were then transported in PreservCyt, a cell collection medium (Hologic, Inc.) for DNA extraction and HPV testing. The study protocol was approved by the Ethics Committee of Pasteur Institute of Morocco and written informed consent was obtained from each participant.

Sample preparation

A 2 ml volume of the preservation solution containing cervical cells was centrifuged at 13000 g for 15 min and pelleted cells were lysed in the digestion buffer (Tris-HCl 10 mM, EDTA 10 mM, NaCl 150 mM and SDS 2%) containing proteinase K (0.1 mg/ml). DNA isolation was performed using standard phenol chloroform method and ethanol precipitation. DNA was then resuspended in ultrapure water and stored at -20 °C until use.

To ensure DNA integrity and the absence of PCR inhibitors, all samples were amplified by PCR using PC04 and GH20 primers specific for human β -globin gene [16]. The amplification mixture contained 6.5 mM MgCl₂, 50 mM KCl, 2.5 U of Ampli Taq DNA polymerase (Roche Molecular Diagnostics), 200 M (each) dATP, dCTP, dGTP, and dTTP, and 50 pmol of each primer (PC04: 5'-CAACTTCATCCACGTTCCACC-3' and GH20: 5'-GAAGAGCCAAGGACAGGTAC-3'). The PCR cycle conditions consisted of an initial denaturation step at 95 °C for 5 min followed by 55 cycles of 35 sec at 94 °C; 30 sec at 60 °C for C-509T and 58 °C for T869C; 30 sec at 72 °C; and a final elongation at 72 °C for 7 min. PCR products were separated by electrophoresis on a 2% agarose gel and samples that generated a 268-bp band were considered positive.

HPV detection

HPV detection was performed by nested PCR using consecutively MY09/11 and GP5+/6+ primers [17, 18]. Primers used to amplify 450 bp fragment of L1 region were MY09: 5'-CGTCCMARRGGAWATGATC-3' and MY11: 5'-GCMCAGGGWCATAAYAATGG-30'. Amplification reaction was performed in 25 μ l reaction mixture containing 1x PCR buffer, 1.5 mM MgCl₂, 100 μ M of each dNTP, 0.2 μ M of forward and reverse

primers, 100 ng genomic DNA and 0.25 U of Taq DNA polymerase (Invitrogen). The mixture was first denatured at 95°C for 5 min, followed by 40 cycles of 94°C for 30 sec, 55°C for 1 min, 72°C for 1 min, and an additional 7 min at 72°C. These PCR products were used as templates for the second PCR to amplify 140 bp using GP5+ and GP6+ primers.

PCR amplification was performed as follows: DNA denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C during 30 sec, annealing at 55°C during 30 sec and extension at 72°C during 30 sec. At the end of the last cycle, the mixture was incubated at 72°C for 7 min. For every reaction, a negative control, in which DNA template was omitted from the amplification mixture, and a positive control, with DNA extracted from SiHa cell line, were included. The products of the amplifications were run electrophoretically on a 2% agarose gel.

HPV genotyping

HPV genotyping was performed by DNA sequencing. Positive PCR products were purified by the ExoSAP ITR clean up system (USB, USA). Sequencing reaction was performed according to the manufacturer's protocol (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, USA). Direct sequencing of amplified PCR products was performed with both forward and reverse primers (GP5+/6+) on an ABI PRISM sequencing apparatus (ABI Prism 3130XL Genetic Analyser, Applied Biosystems, USA). Determination of HPV genotype was done by sequence alignment and comparison with those of known HPV types available through GenBank by using the online BLAST 2.0 software server (<http://www.ncbi.nih.gov/blast>).

Statistical analysis

All analyses were carried out using SPSS 25.0 statistical software package. Categorical variables were presented as frequency and percentage, and chi-square test was used for socio-demographic variables comparison. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess potential associations between the genital HPV infection and potential risk factors. Confounding variables included in the adjusted logistic regression analysis were age, urban-rural habitats and marital status. Differences were considered significant at *P* values less than 0.05.

Results

In this study, 360 women from different regions of Morocco were recruited. These women were attending simple gynecological examination, screening and counseling during medical mobile caravans visits in the Moroccan "sensitization and health promotion" program. The mean age of the recruited women was 45.30 years ranging between 18 to 77 years. The mean age of menarche was 13.57 ± 1.5 ; more than 60% of the women lived in an urban area and most of them were married (92.5%). The majority of women had been pregnant at least one time and the mean number of children was 3.44 ± 2.08 , with a mean age at first birth of 21.42 ± 5.44 . All the participating women had not recurrent genital infections and were ever-smokers.

The overall prevalence of HPV infection was 30.28% (109/360). The distribution of HPV infection according to some potential risk factors is reported in Table 1. Of particular interest, a significant association was observed between HPV infection and age. Indeed, HPV infection prevails in age groups 30 – 35 and 36 – 45 years. Younger and older women presented a moderate and lower frequency of HPV infection with 30% and 15.88% of HPV positive women, respectively

($P < 0.001$). We observed that the prevalence of HPV among those from urban area (41.20%) ($P < 0.001$) positivity was significantly lower in married women (28.52%) than in single/divorced women (51.85%) ($P = 0.011$). HPV infection prevalence was significantly lower among women from rural area (13.88%) than

	Total (n=360)	HPV positive (n= 109)	Infection rate, %	*P value
Age intervals				<0.001
18-25	10	3	30	
25-35	57	21	36.84	
36-45	132	58	43.93	
> 45	161	27	15.88	
Habitat				<0.001
Urban	216	89	41.20	
Rural	144	20	13.88	
Marital status				0.011
Married	333	95	28.52	
Not married	27	14	51.85	

Table 1: HPV prevalence according to potential risk factors

* P value of χ^2 comparing HPV positive and HPV negative women

The highest HPV infection was observed in women from Beni Mellal (55.10%; 27/49), followed by women from Casablanca, Marrakech, Azemmour, Berkane, Goulmim, Haouz and Khmiss Zmamra with 44.89% (44/98), 33.33% (10/30), 30.43% (7/23), 25.92% (7/27), 21.73% (10/46), 7.69% (2/26), and 5.55% (2/36) of infected women, respectively. Of particular interest, no woman from Zawyat Ahansal was HPV positive (0/25).

Overall, 11 distinct genotypes were detected: 7 high-risk (HR) and 4 low-risk (LR) genotypes. The prevalence of

HR-HPV infection was 62.3% (68/109) while that of LR-HPV infection was 37.6% (41/109). Their frequency distribution is shown in Figure 1. HPV16 was the most frequent genotype and was detected in 25.68% (28/109) of infected women. Other HR HPVs, including HPV18, 31, 45, 58, 59 and 82 were identified in 8.25% (9/109), 5.50% (6/109), 1.83% (2/109), 2.75% (3/109), 11.92% (13/109) and 6.42% (7/109), respectively. In this study, all the HPV positive women were found to be infected by a single HPV genotype.

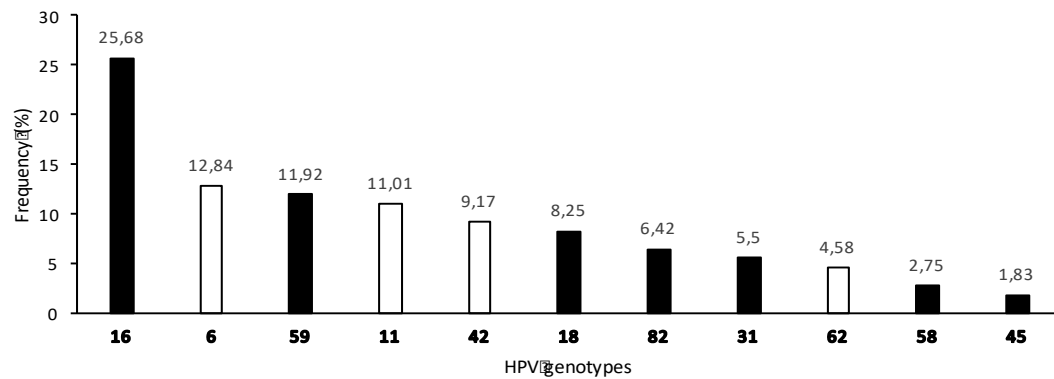


Figure 1: Frequency of high-risk HPV (darks bars) and low-risk HPV (light bars) genotypes identified.

Analysis of the degree of association between genital HPV infection and potential risk factors are presented in Table 2. Our results showed that women aged between 36 – 45 and 30 - 35 years had respectively 3 times and 4 times increased risk of genital HPV infection ($P=0.003$ and $P<0.001$, respectively). Compared to married women, single/divorced had a significant association with HPV infection (OR = 2.69; 95% CI 1.22 – 5.95;

$P=0.014$), however this difference was not statistically significant after adjusting for the covariates (OR = 1.76; 95% CI 0.76 – 4.06; $P=0.18$). Women from rural area were less likely to have HPV infection (OR = 0.23; 95% CI 0.13 – 0.41; $P<0.001$), suggesting that rural habitation may be considered as a protective factor against HPV infection.

	HPV negative n (%)	HPV positive n (%)	Unadjusted OR (CI 95%)	P value	Adjusted OR (CI 95%)	P value
Age intervals						
18-25	7 (70)	3 (30)	2.127 (0.517 – 8.749)	0.296	2.124 (0.451 – 10.011)	0.341 ^a
25-35	36 (63.15)	21 (36.84)	2.895 (1.469 – 5.707)	0.002	3.015 (1.444 – 6.297)	0.003 ^a
36-45	74 (56.06)	58 (43.93)	3.890 (2.272 – 6.660)	<0.001	4.174 (2.361 – 7.379)	<0.001 ^a
> 45	134 (84.11)	27 (15.88)	1.00	-	1.00	-
Habitat						
Urban	127 (58.79)	89 (41.20)	1.00	-	1.00	-
Rural	124 (86.11)	20 (13.88)	0.230 (0.134 – 0.397)	<0.001	0.236 (0.135 – 0.412)	<0.001 ^b
Marital status						
Married	238 (71.47)	95 (28.52)	1.00	-	1.00	-
Not married	13 (48.14)	14 (51.85)	2.698 (1.223 – 5.954)	0.014	1.764 (0.766 – 4.063)	0.183 ^c

Table 2: Analysis of the association between HPV infection and potential risk factors

^a Adjusted by habitat and marital status; ^b Adjusted by age and marital status; ^c Adjusted by age and habitat

Discussion

The main bulk of CC occurs in developing countries with the highest prevalence being registered in countries from Latin America, Asia and Africa [19]. In Morocco, CC is the second most frequent female cancer after breast cancer and represents a major public health problem. Up to now, data on HPV prevalence and HPV genotype distribution among women is limited to two regions: Rabat and Fez, and the data are controversial, suggesting a great difference of HPV distribution in Moroccan regions and highlighting an urgent need for national epidemiological studies to obtain an exhaustive picture of oncogenic HPV prevalence and distribution in Morocco. In this context, this prospective study was planned to evaluate HPV prevalence and HPV genotypes circulating in different regions of Morocco.

In this study, HPV detection was performed by Nested PCR widely reported as the most sensitive method for detection of HPV infection in clinical samples [20]. The overall HPV prevalence was 30.28%. Notably, this prevalence was significantly higher compared to the prevalence reported by Alhamany et al. [14] in Rabat (15.7%) and lower than those reported in women from Fez and its neighborhood by Bennani et al. [15] and Souho et al. [21] which were 42.5% and 43.1%, respectively. Globally, HPV prevalence in Africa in women without CC is estimated to be 22.1%, and is much lower in Europe (8.1%) and Asia (8.0%) [22]. Results obtained in this study are in agreement with those reported in many African countries such as Tunisia (34%), Cameroon (38.5%), Gabon (46%) and Congo (60.4) [22–25].

We detected 11 different HPV genotypes; 7 HR-HPV and 4 LR-HPVs. Among HR-HPVs, HPV 16, 59, and 18 prevail; other HR-HPVs were reported with a

prevalence less than 6%. This distribution is quite different from those reported in women from Rabat and Fez. In women attending the department of children and mothers' pathology of Ibn Sina hospital in Rabat, HPV 16 and 18 were found to be the most prevalent HPV types [14]. However, in women attending the Hassan II University Hospital for cervical pap smears, the most prevalent genotypes were HPV 53, HPV 16 and HPV 35, which were detected with less prevalence [21]. HPV distribution obtained in this study is in agreement with widely reported data and clearly showed that HPV16, 59 and 18, the most oncogenic HPVs, are the predominant genotypes, which encourages the introduction of HPV prophylactic vaccines targeting HPV16 and 18 preferentially.

In Morocco, like in the majority of LMIC, there are continuous changes on cultural habits and sexual behaviors. For a long time, discussion about genital tract diseases was a taboo in Morocco. Moreover, misconceptions and mentality limit HPV testing and largely participate in the dissemination and spread of HPVs in the sexually active population. Many studies were conducted in Morocco regarding HPV knowledge and HPV vaccine acceptability and results converge to a low awareness of HPV infection and a low level of HPV vaccine acceptability among Moroccan adolescents and parents [26–28]. Thus, HPV 16 and 18, the most frequent HPV genotypes in Morocco, are covered by the two commercial vaccines, and efforts must be made by health care providers and educators to increase HPV and HPV vaccine awareness that could increase parental acceptability of the HPV vaccine and consequently a better management of CC and precancerous lesions in Morocco.

Recently, Gardasil 9 was developed and is recommended for individuals from 9 through 45 years of age for the prevention of cervical, vulvar, vaginal and anal cancers caused by human HPV 16, 18, 31, 33, 45, 52, and 58; and genital warts caused by HPV Types 6 and 11. Of particular interest, most HPVs identified in this study are covered by Gardasil 9 prophylactic vaccine.

The association analysis was also performed to evaluate the related risk factors of HPV infection by comparing data between HPV-positive and HPV-negative groups. The highest prevalence of HPV was observed in women aged between 18 and 45 years old. The subjects older than 45 years were at a lower risk of infection with HPV. It is widely accepted that genital HPV infection in women is predominantly acquired in adolescence, and high HPV prevalence appears to differ across geographical regions. Some studies have reported an increase of HPV infection in women older than 45 years [17, 22]. However, a large meta-analysis conducted by Smith and coll. showed inconsistent trends in HPV prevalence by age in older women, with a decrease or plateau of HPV prevalence in older ages in most studies, whereas others showed an increase of HPV prevalence in older ages [29]. Moreover, Masia et al. found that HPV and HR HPV prevalence significantly declined in women older than 46 years in women from Sardinia, Italy [30].

In Morocco, data are also controversial. In Fez and its neighborhood, the high HPV prevalence was observed in older women [21]. However, no significant difference was reported between younger and older women from Rabat [14]. These contradictory results may reflect differences in sexual behavior across cities and/or countries.

It is also noteworthy that women living in urban areas were more likely to be at risk of HPV infection than those living in rural area. This difference in HPV distribution could be related to sexual habits and the level of conservative behavior between rural and urban areas; usually rural communities are more conservative compared to urban communities. Similar results were already reported in Eastern Brazilian Amazon [31] and Shenzhen region of China [32], where HPV prevalence was higher in urban than in rural areas.

The present study is very informative and its effectiveness is mainly related to the multi-sites sample collection. In fact, this is the first study evaluating HPV prevalence and determining HPV genotypes in women all coming, without presumption of cervical lesions, recruited in 9 distinct regions. Moreover, recruitment was done in both urban and rural areas and included both young and elder' women. However, the main limitation of the study is the small sample size, and limited number of regions due to campaigns organization costs.

Conclusion

In conclusion, this study clearly showed that HPV infection is high in Moroccan women. Moreover, these findings show the predominance of HPV 16 and 18 in cervical swabs, and represent together more than 30% of total HPV positive cases, suggesting that the two available vaccines in Morocco, Gardasil and Cervarix, could be of a great advantage in protecting women against HPV infection and limit HPV dissemination in the whole country. Moreover, introduction of Gardasil 9 in Morocco is highly encouraged as this will enhance HPV protection of Moroccan women.

Ethical approval and consent to participate

The Ethics Committee of Pasteur Institute approved the study. All participants gave written informed consent.

Competing interests

The authors declare no conflicts of interest.

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