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Prevalence of HPV and genotype distribution in "catch-up" HPV vaccinated women

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Summary

Purpose of investigation: The aim of the present study was to evaluate the prevalence of HPV and genotype distribution among HPV-vaccinated women. *Material and Methods:* The authors recruited 147 women, which were vaccinated through "catch-up vaccination", five years before this study. The authors analyzed the presence of HPV, genotype distribution, and risk factors for HPV infections. *Results:* The relative prevalence of HPV/DNA was as follows: out of the total 147 tested samples, 19 samples (12.92%) were positive for HPV/DNA, 11 of which (7.48%) were single type HPV infections, and eight (5.44%) tested positive for multiple HPV genotypes. The most frequent genotypes were: 16, followed by 35, 56, and 31. Low risk genotypes 6 and 11 were present together in one case. *Conclusion:* The results showed a decrease in HPV prevalence 16/18 in vaccinated women through a catch-up vaccination, suggesting good effects of the previous HPV immunization program.

Key words: HPV infection; HPV vaccine; "Catch-up" vaccination.

Introduction

Prophylactic vaccines could have an important impact on public health. Since 2006 two prophylactic HPV vaccines are available: the bivalent (that protect against HPV 16 and 18) and quadrivalent vaccine (against 6, 11, 16, and 18). Both available vaccines are targeted against 16 and 18 high-risk genotypes and it is expected to observe a decrease in the prevalence of these types. The quadrivalent vaccine can prevent HPV6- and HPV-11-associated cervical lesions, genital warts, and, possible, recurrent respiratory papillomatosis. On the other hand, recent results suggest that the bivalent vaccine may offer greater cross-protection against high oncological risk HPV 31, 33, 45, 52, and 58 and, potentially, longer duration of protection [1]. In order to evaluate the short impact of the vaccine, the monitoring of HPV prevalence and genotype distribution in population prior to, and after vaccine introduction is essential. It is possible to observe a change not only in 16 and 18 genotypes prevalence, but also in other subtypes because of the cross-protection or potentially by replacement by other genotypes.

However, considering that most government-funded HPV immunization programs target only pre-teen girls and that the latency between HR-HPV infection and development of invasive cancer is long (10-15 years), it is expected that more than a decade will pass before a reduction in mortality from cervical cancer will be observed in most countries [2]. A notable exception might become evident in countries with successful "catch-up" programs in place. For example, Australia was the first country to introduce a fully-funded national immunization program for women up to the age of 26, and as a result of high vaccine uptake in the "catch-up" age range, it was also the first country to report a significant decline in the rate of high-grade precancerous lesions [3, 4].

Because Romania has the highest incidence of cervical cancer in Europe, in 2008 a HPV vaccination campaign was introduced targeting 10-11-year-old girls. Statistics from 2008 revealed that only 2.5% of the 110,000 eligible girls in the target group were vaccinated [5]. Thus, a re-launching of the vaccination campaign was planned for 2009-2010, targeting girls between 12- and 14-years-old and because many doses were about to expire, the "catch-up" population was included [6]. Because of the vaccination of women over 16 years in the present country it is possible to evaluate the changes in HPV prevalence and type of virus prior and after vaccination. Because of this group which was vaccinated starting 16 years, the present is one of the fewest country in the world where we can evaluate the short-term effect of the vaccination in term of observing how the vaccine change the prevalence or type of HPV infection in the population.

This study assessed the first effects of catch-up vaccination by evaluating the prevalence of HPV infection in a group of HPV vaccinated women. Taking into account that the distribution of HPV can vary according to geographic

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Table 1. — *Socio-demographic characteristic of the participants.*

Variable	Number	Percentage (%)
Age		
17-25	16	10.88
25-35	68	46.25
over 35	63	42.85
Marital status		
Single	53	36.05
Married	74	50.34
Concubinage	13	8.84
Divorced	7	4.76
Education level		
Primary	12	8.16
Secondary	37	25.17
University degree	98	66.66
Number of children		
No children	65	44.21
1	41	27.89
2-3	20	13.60
> 3	4	2.72
Occupation		
Employee	88	59.86
Student	17	11.56
Social aid	4	2.72
Without occupation	38	25.85

regions, the authors also compared the distribution type of HPV among vaccinated and non-vaccinated women in the same geographic region.

Materials and Methods

The authors performed a cross-sectional study in the North Eastern region of Romania. They recruited 147 women, which were vaccinated through "catch-up vaccination" in 2010-2011. Subjects were invited to participate to this study via invitation letters or by telephone (by gynecologists and general practitioners) between September 2015 and January 2016. The Bioethical Committee of the "Grigore T. Popa" University of Medicine and Pharmacy approved the study. Written informed consent was obtained from all the participants, after they were informed about the study. Each woman completed a questionnaire concerning possible cofactors for cervical cancer (e.g. smoking, genital co-infections, oral contraceptive use, number of sexual partners), and information regarding their knowledge about HPV and HPV vaccine. The participants were examined and evaluated for the presence of genital warts, and HPV sample was collected. The gynecologist collected cervical cells in PCR Cell Collection (HPV-SCK code 03-33) from all the women, and then kept at 4ºC till processing (1-7 days). DNA extraction was made with QuickGene DNA tissue kit S in the first 15 days after the samples were collected. Specimens were analyzed using a general primer based polymerase chain reaction (PCR) and genotyped for 16 high-risk and low-risk HPV-strain-based multiplexed genotyping. The technique was validated through the use of positive and negative controls (primers against β-globine gene) at each shift. The controls are necessary in order to discover if the swab is prepared correctly, as an amplification control for each individually processed specimen and to identify possible reaction inhibition. All women with HPV/DNA positive test had a Papanicolaou smear and a colposcopy.

Table 2. — *The distribution of HPV genotypes.*

HPV type	Genotype risk	Frequency	Percentage (%)
Negative		128	87.07
16	HR	7	4.76
35	HR	5	3.40
56	HR	4	2.72
31	HR	3	2.04
59	HR	3	2.04
33	HR	2	1.36
45	HR	2	1.36
52	HR	1	0.68
11	LR	1	0.68
6	LR	1	0.68

HR= high-risk, LR=low-risk types.

Results

The mean age of the study population was 34.08 (21-49) years; 16 women (10.88%) were aged under 25 years, 68 (46.25%) between 25 and 35, and 63 (42.85%) over 35-years-old. One hundred thirteen participants (76.87%) were from urban area and 34 (23.12%) from rural area. The main socio-demographic characteristics are presented in Table 1. The majority of women (81.60%) received the quadrivalent vaccine, while the remaining received the bivalent vaccine. Because HPV test was not covered by medical insurance, it was not performed before vaccination. The time between vaccination and the present study was between five and six years.

The following prevalence of HPV/DNA was identified in thus study: out of the total 147 tested samples, 19 samples (12.92%) were positive for HPV/DNA. 11 samples (7.48 %) were single HPV type infections, and eight samples (5.44%) tested positive for multiple HPV types. Multiple infections were produced by two genotypes per sample in six cases (4.08 %) or three genotypes per sample in two cases. Multiple infections were only with HR types in seven cases (4.76 %) or a combination of LR types and HR types in one case (0.68%). The distribution of HPV types is presented in Table 2. All positive cases had at least one HR genotype. The most frequent was 16, followed by 35, 56, 31, 59, 33, 45, and 52 (Table 2).

The prevalence of HPV genotypes included in the bivalent/ quadrivalent vaccine was: HPV 16: 4.76% (7/147), HPV 6: 0.68% (1/147), and HPV 11: 0.68% (1/147). HPV 18 was not identified among samples in this study. Even in cases with multiple infections, type 16 was the most frequently encountered.

The distribution of HPV infections according to the age groups was: under 24 years age three cases, 25-34 years eight cases, over 35 years eight cases (Table 3).

Table 3. — *Positivity rates of HR-HPV, and LR-HPV stratified for age groups among women with HPV.*

Age group (years)	HPV positive test	HR-HPV	LR-HPV
< 24	3 cases	3 cases	0
25-35	8 cases	3 cases	1
> 35	8 cases	8 cases	0
Total	19 cases	19 cases	1

HR= high-risk, LR= low-risk.

Table 4. — *Pap smear results among women with HPV positive tests.*

Pap smear results	Single infection	Multiple infection	HR-HPV	LR-HPV
Inflammatory	4	2	6	0
ASCUS	2	3	5	1
LSIL	0	2	2	0
HSIL	0	1	1	0
Normal	5	0	5	0
Total cases	11	8	19	1

ASCUS= atypical cells of undetermined significance.

The present authors correlated the presence of HPV with risk factors for HPV infections and cervical cancer: use of oral contraception, smoking, early onset of sexual activity, multiple sexual partners. Of all 19 HR-HPV positive women, four (21.05%) declared the use of oral contraceptive, eight (42.10%) reported cigarette smoking, and six (31.57%) reported more than three sexual partners. Of all 128 the DNA/HPV negative women, 25 (19.53 %) declared the use of oral contraceptives, 28 (21.87%) reported cigarette smoking, and 30 (23.43%) reported more than three sexual partners.

During clinical examination only in one case genital warts were present and in this case both types 6 and 11 were present, the patient being vaccinated with bivalent vaccine. The authors identified the following Pap smears results among women with HPV infection: inflammatory six cases (31.57%), ASCUS: five cases (26.31%), LSIL: two cases (10.52%), and HSIL: one case (5.26%). In 5/19 (26.31%) cases positive for HR-HPV, the result of conventional smear was reported as "normal" (Table 4). In two cases colposcopy and biopsy confirmed a CIN I lesion and in one case a CIN II lesion.

Discussion

Vaccination is among the most successful and less costly of all public health interventions. In non-vaccinated population the prevalence of HPV infections may vary according to geographic area. Large population-based studies in Denmark and the US found an HPV prevalence of 50.2% and 53.8% in non-vaccinated women aged 20-24 years, respectively [7, 8]. The geographic area where the present authors performed this study has a population of 4.5 million people. In this geographic region, two studies regarding incidence and distribution of HPV types were performed, both in non-vaccinated population. The first one was performed between 2007-2009, and analyzed 152 women with an average age of 40 years [9]. HPV infections were present in 67.76% of cases and 75% of cases had high-risk genotypes. The most prevalent oncogenic genotype was HPV 16 (43.12%), followed by HPV 18 (10.34%), and HPV 31 (10.34%). The other study found a prevalence of HPV infection of 37.4% in a sample of 514 women with a median age of 36.5 (17-84 years) years [10]. In this study, the most frequent types were: 16 (10.5%), 53 (5.44%), 51 (5.05%), 52 (4.08%), 18 (2.91%), and 31 (2.73%). Both studies report high prevalence of HPV in a non-vaccinated population, HPV 16 being the most prevalent. In both studies genotype 18 had a low prevalence and this could represent a particularity of genotype distribution in this geographic area. In the present study performed in the same geographic region among vaccinated women, the overall prevalence of HPV-HR was 12.92% and the most frequent types encountered were: genotype 16, followed by 35, 56, 31, 59, 33, 45, and 52. Among the participants in this study, HPV genotype 16 and 18 prevalence was significantly lower in vaccinated than in non-vaccinated women of the same geographic area. These findings are in line with recently published studies from Australia and the US [3, 4]. The presence of genotypes 16 among vaccinated women in the present study is likely due to the fact that most vaccinated women received the vaccine after sexual onset and in many cases an HPV test before vaccination was not performed. Thus, it is possible that in some cases the infection with HPV types included in the vaccine to be present at the moment of immunization and explains the prevalence of types 16 after immunization. These findings support the recommendation to vaccinate early in adolescence and before sexual onset. In fact, vaccination of HPV16/18 DNA positive women does not enhance clearance of the viral infection [11]. It is thus important to promote vaccination at an age when the vaccine is most effective immunologically and when uptake is likely to be high.

No patient in the present study had clinical vulvo-vaginal neoplasia or condyloma. Eighty-seven percent of the women in this study were vaccinated with quadrivalent vaccine, which offer immunization for low-risk types responsible for genital warts. The authors found that prophylactic HPV vaccination had a good efficacy against vulvovaginal neoplasia and condyloma attributed to HPV types 6 and 11 at least four years after immunization. This confirms the results of previous studies with shorter follow-up times and provides evidence of longer duration of protection with no signs of waning protection. The quadrivalent vaccine against HPV offers not only 100% protection against anogenital disease in women who have not been exposed to HPV prior to vaccination, but it also reduces the number of anogenital lesions

in women who may have been infected with HPV [12].

Consistent with other data, a higher number of lifetime sexual partners are an independent risk factor for HPV infection. In the present study, 31.57% from HPV positive women declared more than three sexual partners in comparison with 23.43% of the negative HPV women. Several studies demonstrated that smoking interferes in the increase of HPV infection prevalence and in an increased risk of CIN and cervical carcinoma [13, 14]. In the present study the percentage of active smokers was greater in HPV positive women in comparison with HPV negative women. In addition, a lower educational status was associated with a higher HPV prevalence. Lower educated women were under-represented in this study, but this finding is supported by a large study from the US, where a lower educational status was a predictor of HPV detection [15].

The present study confirms that vaccination will ultimately change the natural history of HPV disease by reducing the influence of the highly oncogenic types HPV 16 and 18, not only in girls vaccinated before sexual onset, but also in "catch-up" vaccinated group. However, even if a level of cross protection against other genotypes is proved, the immunization alone cannot completely prevent cervical cancer. Although the vaccination decreases the prevalence of HPV, HPV infection is still present in vaccinated population and screening is still necessary. Vaccination and screening act complementarily and synergistically, and constitute to date the new standards of disease prevention [16].

Some limitations of the present study need to be mentioned. Before "catch-up" vaccination in this country, not all women had an HPV test and it is possible that some of the participants already had the infection at that time. Because low educated women were under-represented in this study, it would be interesting in the future to study a more heterogeneous group.

Conclusion

The study provides a useful baseline HPV prevalence estimate shortly after the introduction of prophylactic HPV vaccination in Romania. The results showed a decrease in HPV prevalence 16/18 among vaccinated women through "catch-up" vaccination, suggesting good effects of previous immunization campaign. These finding enhance the usefulness of catch-up vaccination but reinforce the recommendation to vaccinate girls in early adolescence and before sexual debut when vaccine is immunologically most effective and when uptake is likely to be high.

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