

## Prevalence of high-risk human papillomavirus and distribution of HPV16/18/45 types in Albanian young women before HPV vaccination

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### Abstract

**Aim:** To estimate the prevalence of high-risk human papillomavirus (hr-HPV) infection among 18-24 year old women, prior to implementation of prophylactic HPV vaccination in Albania, and to determine 16/18/45 HPV genotypes distribution in order to assess the potential benefit of HPV vaccination.

**Methods:** A total of 382 women, aged 18-24 years, were screened with Digene Hybrid Capture 2 HPV DNA Test for 13 hr-HPV types at the Molecular Biology Laboratory of the national Institute of Public Health in Tirana, Albania. Genotyping for hr-HPV 16/18/45 types was performed to positive women.

**Results:** Out of 382 samples, 95 (24.87%) [95% CI, 19.87 - 29.87] were hr-HPV positive. The prevalence of infection with HPV 16 was 21.05% (95% CI=16.0%-26.1%), whereas the prevalence of infection with HPV 18 was 10.52% (95% CI=5.5%-15.5%). The hr-HPV prevalence in women with abnormal cytology was 27.4%; nonetheless, it was no significantly higher than in women with normal cytology (57.9%).

**Conclusion:** The hr-HPV prevalence in young women in Albania was higher than among older women, using the same molecular method and the same laboratory. Findings of this study provide useful baseline data on pre-vaccination distribution of HPV genotypes in target populations of Albania.

**Keywords:** cervical cancer, genotype distribution, high-risk types, human papillomavirus, prevalence.

## Introduction

Infection with high-risk types of human papillomavirus (hr-HPV), very common in sexually active women, is a necessary cause for cervical cancer (CC) development (1,2). In most cases, genital infection with HPV is transient. Only persistent infection by hr-HPV types is likely to develop cervical lesions (3-7). Approximately 71% of all cervical cancers are caused by HPV16 and 18 (8-10). Two prophylactic vaccines targeted to provide protection against HPV infection have been licensed in European Union: the bivalent vaccine containing L1 virus-like particles of HPV types 16 and 18, and quadrivalent vaccine containing L1 virus-like particles of HPV types 16, 18, 6, and 11, preventing premalignant genital lesions, cervical cancer and genital warts (10-13). Baseline information on pre-vaccination distribution of HPV genotypes in target populations is essential for designing, monitoring and evaluating immunization strategies. Pre-vaccination prevalence is relevant to estimate vaccine effectiveness against the HPV-vaccine types, to evaluate the cross-protection of the vaccines, and to monitor over time the relative frequency of genotypes under the selective pressure of the vaccines.

In Albania, self-paid not mandatory vaccination against HPV with licensed bivalent vaccine is available. But, there is very limited amount of data on HPV prevalence and type-specific distribution in younger ages. Investigation of the HPV infection prevalence is the basis for developing prophylactic strategies against cervical cancer, especially for young women.

To characterise HPV infection prevalence in Albania prior to implementation of prophylactic HPV vaccination and to determine local HPV genotypes specifics in order to assess the potential benefit of HPV vaccination in Albania, starting has inviting 18-24 years old women for HPV screening. This study aimed to estimate the pre-vaccination prevalence of cervical infection and distribution of local hr-HPV types 16/18/45 among sexually active

young Albanian women.

## Methods

### *Study population*

A sample of 382 sexually active women was randomly selected from the Molecular Biology Laboratory register (Institute Public Health in Tirana) during April 2015 - June 2016 period of time. The inclusion criteria were: women aged between 18 and 24 years. Exclusion criteria were: attendance after an atypical/abnormal cytology result, menstruating or pregnancy at presentation. A standardized questionnaire was obtained from all women for demographic, epidemiological and personal history data.

### *Specimens and data collection*

Specimens were self-collected with the Digene Female Swab Specimen Collection Kit (Qiagen, Gaithersburg, MD) for HPV testing. The swab was transferred into a vial containing 1 mL Specimen Transport Medium (STM). Samples were then stored at -20°C, till processing for routine hr-HPV DNA testing.

### *HPV testing and genotyping*

The HPV test was performed using Hybrid Capture 2 (HC2 High-Risk HPV DNA, Qiagen; Gaithersburg, Germany) according to the manufacturer's instructions. It is a hybridization assay that detects the presence of HPV DNA using cocktails of RNA probes and an amplified, chemiluminescent signal. The high-risk group of probes, designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 were used. The assay is calibrated on a positive cut off (PCO) of 1 pg/ml of HPV DNA. Samples were considered positive when the ratio between the Relative Light Units (RLU) of specimen and the PCO attained or exceeded the value of 1.0.

All cervical specimens positive for hr-HPV were retested with *digene* HPV Genotyping PS test

(Qiagen, Gaithersburg, MD) - a reflex test intended for the individual qualitative detection of high-risk HPV types 16, 18, and 45. This test has an analytical sensitivity (1 pg/mL) similar to that of the HC2 test.

### Statistical analysis

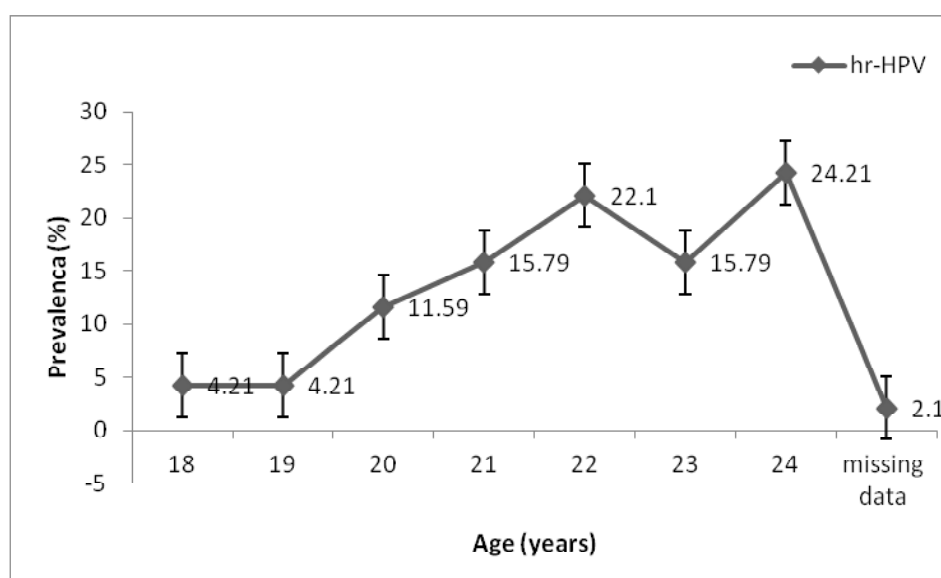
Statistical analysis was performed using the SPSS software package version 19.0. We estimated the prevalence of infections with 13 hr-HPV types with exact 95% confidence interval (CI). Prevalence was defined as number of women positive for any HPV infection at the time of the first screened by HPV test. In this study continuous variables are presented as range (mean) and Standard Error (SE), and qualitative variables as frequencies and percentages. To assess the association between the prevalence of infection with any hr-HPV type, as well as infection with HPV16 and HPV18 and age groups at time of the HPV test, Chi-square test for linear trend and Fisher's exact test was calculated. Also to assess

the association between the prevalence of infection with hr-HPV type and cytology results a univariate logistic regression was calculated. Level of statistical significance was set at  $p < 0.05$ .

### Results

From 2015 to 2016, a total of 382 females aged 18-24 years were tested for hr-HPV infection at Molecular Biology Laboratory in the National Institute of Public Health. Mean age of study participants was 21.92 years (median age: 22 years). The prevalence of infection by age is reported in Figure 1. The prevalence of infection with hr-HPV type was 24.87% (95%CI=19.8%-29.87%). There was an overall significant increase in hr-HPV prevalence with age. Women over 20 years of age were 1.47 time at higher risk of being infected by hr-HPV without a confidence level (95%CI=0.83%-2.6%;  $P=0.17$ ) in comparison with women below 20 years (26.43% and 19.6%, respectively).

**Figure 1. Prevalence of infection with high-risk HPV types with 95% confidence intervals according to age**



Results from genotyping test showed 34.73% (95%CI=29.3%-39.7%) positivity for HPV 16/18/45 types. Among positive women, HPV16 was the

most prevalent type (21.05%), followed by HPV18 (10.52%); the cumulative rate of 16 and 18 was 34.73%.

The distribution of infections with individual hr-HPV types among women infected with any hr-HPV type, overall and according to the cytology result is shown in Table 1.

From the 382 women tested for hr-HPV, we have a known cytology result for only 23.6% (90/382) of them. The pap-test results were available for 84 from 95 samples resulted positive for hr-HPV.

**Table 1. Prevalence of infection with high-risk HPV types, overall and according to cytology result among 382 women screened for hr-HPV**

HPV types	Cytology result											
	All women (N =382)			Normal			Atypical cells*			LSIL†		
	N	Prevalence (%)	95% CI	N	Prevalence (%)	95% CI	N	Prevalence (%)	95% CI	N	Prevalence (%)	95% CI
Any hr-HPV	95	24.87	19.87-29.87	55	57.9	52.4-63.0	16	16.84	11.8-21.84	10	10.5	5.5-15.5
HPV16 or/and 18‡	33	34.73	29.3-39.7	ND§			NA			1	10	5-15
HPV 16/18/45	1	1.05	0-6.0	ND			NA			ND		
HPV16	20	21.05	16.0-26.1	15	27.27	22.27-32.27	4	25	20-30	1	10	5-15
HPV18	10	10.52	5.5-15.5	1	1.81	0-6.81	NA			ND		
HPV45	2	2.1	0-7.1	1	1.81	0-6.81	NA			ND		

\*Atypical cells: atypical squamous cells of undetermined significance (ASC-US) (11 women), atypical squamous cells HSIL (ASC-H) (1 woman);

†LSIL: low-grade squamous intraepithelial lesions;

‡Women with only high-risk HPV16 infection or only high-risk HPV18 or infection with both;

§Not detected.

Cytology was normal in 57.9% of positive hr-HPV samples (55/95) and abnormal in 27.4% (26/95) samples: 15 ASCUS/AGUS (atypical squamous/glandular cervical cells of undetermined significance), 10 LSIL, 1 ASCH. The proportion of hr-HPV positivity was no higher in women with abnormal cytology (26/95, 27.4%) than in women with normal cytology (55/95, 57.89%) but it is found a significant level [odds ratio 3.7; 95% CI (2-7.1),  $P < 0.001$ ].

HPV16 was the most frequent virus type in women with normal cytology, detected in 22.1% of them. From the 10 women with LSIL only four of them were hr-HPV positive and two different types (16, 16/18) were detected respectively.

Among 55 women with normal cytology the overall hr-HPV prevalence varied according to age from

20% below 20 years to 37.89% above 20 years ( $P < 0.01$ ). HPV 16/18/45 types were detected in 30.9% of women with normal cytology result, 25% with ASCUS, 20% with LSIL No significant level was found between them ( $P > 0.05$ ).

## Discussion

This study is the first to estimate the prevalence of cervical infections with 13 hr-HPV types in Albanian women aged 18–24 years before the introduction of HPV vaccination in Albania. We found a prevalence of 24.87%, higher than what detected for older women (16.7%), using the same molecular method and the same laboratory in Albania (14). Similar to global HPV burden, and HPV type circulation in Albania, cervical infection with oncogenic HPV16 type was most common (14).

Our results provide useful baseline data for adopting and monitoring an appropriate HPV vaccination program in Albania and for development in the future of cervical cancer screening strategies for

this target population. It should be mentioned the fact that our sample, being in a small number, may not be entirely representative of Albania's general population of females aged 18–24 years.

**Conflicts of interest:** None declared.

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