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Pre-vaccination prevalence of high-risk human papillomaviruses (HPV) in women from Kosovo and their related sociodemographic characteristics

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ABSTRACT

Objectives: Kosovo's current health care system does not support organized nationwide cervical cancer screening and human papillomavirus (HPV) vaccination programs. To date, no reliable data are available on cervical cancer incidence and mortality in Kosovo, or on high-risk HPV (HR-HPV) prevalence and HPV type distribution. Our aim is to determinate the pre-vaccination prevalence and distribution of HR-HPVs and to assesses the associations between sociodemographic characteristics and increased risk of HPV infection in women from Kosovo.

Material and methods: Detection of HR-HPV DNA in cytologically evaluated cervical smears was performed using a clinically validated Abbott RealTime High Risk HPV test, Roche Linear Array HPV Genotyping Test, HPV52 type-specific real-time PCR and an in-house GP5+/GP6+/68 PCR.

Results: The crude overall prevalence of any of the HR-HPVs was estimated at 13.1% (26/199; 95% confidence interval (CI): 9.1–18.5%), with HPV16 being the most common type (7/26, 26.9%), followed by HPV31 and HPV51, each detected in 4/26 (15.4%) cervical specimens, HPV18, detected in 3/26 (11.5%) specimens, HPV52 and HPV66, each detected in 2/26 (7.7%) specimens, and HPV33, HPV45, HPV56, and HPV58, each detected in a single (3.9%) specimen. Women over 40 (OR = 0.36), older than 18 at sexual debut (odds ratio (OR) = 0.28), those that had delivered at least one child (OR = 0.32), and those that had a history of pregnancy termination (OR = 0.39) were at lower risk for HPV infection.

Conclusion: Because more than 70% of cervical precancerous lesions could have been prevented in Kosovo using nationwide HPV-based cervical cancer screening and HPV vaccination, it is of outmost importance to implement both programs in the national health care system as soon as possible.

Key words: cervical cancer, high-risk human papillomaviruses, Kosovo, prevalence, vaccination

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INTRODUCTION

Cervical cancer is the fourth most frequent cancer in women; in 2012 and 2015 there were an estimated 530,000 and 526,000 new cases worldwide, respectively, and it was responsible for 266,000 (7.5% of all cancer deaths) and 239,000 (6.5% of all cancer deaths) deaths [1, 2]. In 2015, one in 68 (1.5%) women developed cervical cancer before age 79, with the odds being higher in low-income countries, where one in 24 (4.2%) women developed cervical cancer, in comparison to high-income countries, where one in

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115 (0.9%) women developed cervical cancer during their lifetime [2]. Between 2005 and 2015, age-standardized incidence rates of cervical cancer decreased globally; however, the decline was less prominent in low-income countries [2]. Persistent infection with certain types of human papillomaviruses (HPV) is a necessary, but non-sufficient, etiological factor of cervical cancer [3, 4]. To date, 206 HPV types have been identified and officially recognized by the International Human Papillomavirus Reference Center, located at the Karolinska Institute in Stockholm, Sweden (http://www.hpvcenter.se/html/refclones.html). According to their clinical relevance, approximately 40 HPV types from the genus *Alphapapillomavirus* (*Alpha*-PV) are roughly subdivided into high-risk (HR) and low-risk (LR) HPV types. HR-HPVs (Group 1 carcinogens: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV59) are etiologically associated with more than 98% of cervical cancers, 70 to 90% of anal and vaginal cancers, 40% of vulvar cancers, 47% of penile cancers, and 25 to 30% of oropharyngeal cancers. In addition, HPV68 is considered probably carcinogenic to humans (a Group 2A carcinogen), and HPV26, HPV30, HPV34, HPV53, HPV66, HPV67, HPV69, HPV70, HPV73, HPV82, HPV85, and HPV97 are classified as possibly carcinogenic to humans (Group 2B carcinogens). Low-risk HPV types (mainly HPV6 and HPV11) are associated with more than 90% of anogenital warts and laryngeal papillomas [3, 4].

Worldwide, the age-adjusted prevalence of HPV infection in women with normal cervical cytology is estimated at 10.4%, ranging from 8.1% in Europe to 22.1% in Africa [5]. Several global meta-analyses have suggested that the most prevalent HPV types in normal cervical cytological specimens are HPV16, HPV18, HPV31, HPV52, and HPV58 [5–7]. Moreover, whereas HPV16 and HPV18 are etiologically associated with 71% of cervical cancer cases, this percentage rises to more than 90% when including infections with HPV6, HPV11, HPV31, HPV33, HPV45, HPV52, and HPV58 [4, 8, 9]. Because HPV prevalence and HPV type distribution vary greatly across different populations and geographical regions, regional HPV prevalence and type distribution studies are important before implementing HPV-based cervical cancer screening and HPV vaccination in particular countries or regions [5, 8].

Kosovo is a small low-income country with a population of 1.7 million in southeastern Europe that has recently experienced war and postwar difficulties. Kosovo's current health care system does not support organized nationwide cervical cancer screening and HPV vaccination programs. In contrast to other countries in the region (e.g. Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Macedonia, Montenegro, Romania, and Serbia), no reliable data are available on cervical cancer incidence and mortality in Kosovo, or on HR-HPV prevalence and HPV type distribution. Thus, the objectives of this study were to determine for the first time the pre-vaccination prevalence and distribution of HR-HPVs and to assess the associations between particular sociodemographic characteristics and increased risk of HPV infection in women from Kosovo.

MATERIAL AND METHODS

Specimen and data collection

A total of 199 women, 18 to 63 years old, attending the outpatient unit of the Department of Gynecology and Obstetrics, from August 2015 to February 2016, were enrolled and had a valid sample for HPV testing. The cohort represents female population of Kosovo attending the screening for cervical cancer. Namely outpatient clinic of the University Clinical Centre of Kosovo (UCCK), based in Prishtina, where our study was performed is the only public institution in Kosovo where the screening for cervical cancer and PAP-smear testing are offered to patients in whole country. Patients included in the study were randomly selected from all patients visiting the outpatient Clinic, were the main purpose of the visit of the women was screening for cervical cancer or just routine gynaecological examination. All of the women provided written informed consent and completed a questionnaire on sociodemographic characteristics. During a gynecological examination, one cervical smear was obtained for cytological examination, and another cervical smear was stored in 1 ml of Digene Specimen Transport Medium (STM) (Qiagen, Hilden, Germany) at −20 °C for subsequent HPV typing.

Detection of HPV DNA and HPV typing

Detection of HR-HPV DNA was performed using a clinically validated RealTi*m*e High Risk HPV test (RealTi*m*e) (Abbott, Wiesbaden, Germany), which enables concurrent individual typing for HPV16 and HPV18, as well as pooled detection of 12 other carcinogenic HPV types (HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68), and also includes an internal process control for sample adequacy, DNA extraction, and amplification [10, 11]. In addition to several other transport media [10], the assay has also been clinically validated for use with cervical specimens collected with Digene STM (Qiagen) [12]. In specimens positive for other carcinogenic HPVs, HPV typing was subsequently performed using the Linear Array HPV Genotyping Test (Roche LA) (Roche Molecular Diagnostics, Branchburg, NJ) and, if necessary, also with an HPV52 type-specific real-time PCR assay and an in-house GP5+/GP6+/68 PCR assay, as described previously [11, 13, 14].

Statistical analyses

Univariate and multiple logistic regression analyses were used to assess the associations between sociodemographic characteristics and overall HPV infection. A likelihood ratio

test was used to investigate the associations between sociodemographic characteristics and HR-HPV types. Associations with $p < 0.05$ were treated as statistically significant. Analyses were performed with SPSS, version 23.

Ethical statement

This study was performed in compliance with the Declaration of Helsinki and was approved by the Ethics and Professional Board of the Ministry of Health of the Republic of Kosovo (consent number 05-2826). All women included in the study provided written informed consents and only their sociodemographic characteristics were available to all researchers, preserving patients' anonymity.

RESULTS

A total of 199 women, 20 to 63 years old, with a mean age of 41.8 years (*SD* ± 9.3 years) were included in the study (Tab. 1). The majority of women had graduated from a secondary school (107/199, 53.8%), were working as housewives or farmers (143/199, 71.9%) and were married (176/199, 88.4%), were a part of a small family (only parents and their children: 141/199, 70.9%) with a sufficient income (104/199, 52.3%), were at least 18 years old at the time of first sexual intercourse (168/199, 84.4%), had given birth to at least one child (178/199, 89.4%), had had at least one pregnancy terminated (100/199, 50.3%), had a regular menstrual cycle (126/199, 63.3%), were not using contraception (166/199, 83.4%), and were non-smokers (149/199, 74.9%) (Tab. 1). The vast majority of women (165/199, 82.9%) had a normal cytological result (negative for intra-epithelial lesion or malignancy, NILM). Atypical squamous cells of undetermined significance (ASCUS) were detected in cervical specimens of 30/199 (15.1%) women and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), and cervical cancer were detected in two women, respectively (Tab. 1).

Detection and typing of HPV infection

The crude overall prevalence of HR-HPV infection with any of the targeted 14 HPV types was estimated at 13.1% (26/199, 95% confidence interval (95% CI): 9.1–18.5%) (Tab. 2). As shown in Table 3, infections with HPV16 were overall the most common, followed by HPV31 and HPV51, and HPV18. Similarly, HPV16 was also the most frequently detected HPV type in patients with NILM, followed by HPV31 and HPV51 (Tab. 3). Patients with ASCUS were most frequently infected with HPV18 (Tab. 3).

Both cervical cancer cases determined by the cytological examination were additionally confirmed using histopathology. Even though no multiple HR-HPV infections were determined in our study population, using Roche LA and an in-house GP5+/GP6+/68 PCR assay, single HPV16 and HPV66-positive cervical specimens were additionally positive for HPV84 and HPV67, respectively, two HPV51-positive cervical specimens were additionally positive for HPV53 and HPV62, respectively, and the HPV58-positive cervical specimen was additionally positive for HPV6 and HPV73.

Association between sociodemographic characteristics and HPV infection

As shown in Table 4, using univariate logistic regression analysis to investigate potential differences in the sociodemographic characteristics of HPV-negative vs. HPV-positive patients, statistically significant associations with HPV infection were found only for women's age ($p = 0.015$), age at the time of first sexual intercourse ($p = 0.006$), delivery ($p = 0.026$), and history of pregnancy termination ($p = 0.033$). Despite the fact that the prevalence of HPV infection was the highest in women 30 to 39 years old (21.2% (11/52); mean: 20.8%; standard deviation SD: ± 6.4), followed by women 20 to 29 years old (19.1% (4/21); mean: 19.0%; SD: ± 10.5), 40 to 49 years old (9.6% (8/83); mean: 9.6%; SD: ± 4.2), and over 50 (7.0% (3/43); mean: 7.0%; SD: ± 5.8) (Fig. 1), the differences were not statistically significant ($p = 0.127$). However, women over 40 had lower odds for HPV infection (odds ratio (OR) = 0.36; 95% CI: 0.15–0.84) (Tab. 4). Moreover, lower odds for HPV infection were also observed among women that were at least 18 years old at sexual debut (OR = 0.28; 95% CI: 0.11–0.69), had given birth to at least one child (OR = 0.32; 95% CI: 0.11–0.91), or had a history of pregnancy termination (OR = 0.39; 95% CI: 0.16–0.95) (Tab. 4). Due to the small number of units in the category of the dependent variable (HPV infection), only three independent variables (age at inclusion in the study: up to 40 years old and over 40, age at first sexual intercourse: below 18 and at least 18 years old or older, and history of delivering at least one child), were included in the multiple logistic

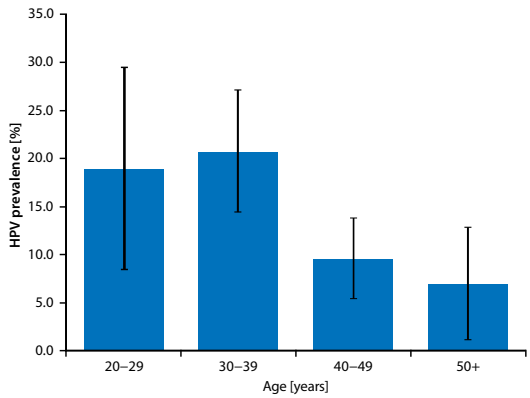

Figure 1. Age-specific HPV prevalence with standard deviation among 199 women from Kosovo included in the study

Table 1. Socio-demographic characteristics of women included in the study and the results of cytology	
	N = 199 (%)
(Mean) age (SD)	41.8 (9.3)
< 40 years old	79 (39.7)
> 40 years old	120 (60.3)
Education	
Finished elementary school or less	51 (25.6)
Finished secondary school	107 (53.8)
More than secondary school	41 (20.6)
Employment status	
Employed	51 (25.6)
Housewife or farmer	143 (71.9)
Student	5 (2.5)
Marital status	
Married	176 (88.4)
Divorced/widow	13 (6.5)
Single	10 (5.0)
Family size	
Single	4 (2.0)
Small family (parents&children)	141 (70.9)
Large family (parents, children and other family members)	54 (27.1)
Family income*	
Insufficient	54 (27.1)
Sufficient	104 (52.3)
Good	41 (20.6)
Age at first sexual intercourse	
< 18 years old	31 (15.6)
≥ 18 years old	168 (84.4)
Delivery	
Yes	178 (89.4)
No	21 (10.6)
Pregnancy termination	
Yes	100 (50.3)
No	99 (49.7)
Menstrual cycle	
Regular	126 (63.3)
Irregular	40 (20.1)
Postmenopausal	33 (16.6)
Contraception use	
Yes	33 (16.5)
No	166 (83.4)
Smoking	
Yes	50 (25.1)
No	149 (74.9)
Cytology	
Negative for intra-epithelial lesion or malignancy (NILM)	165 (82.9)
Atypical squamous cells of undetermined significance (ASCUS)	30 (15.1)
Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)	2 (1.0)
Cervical cancer	2 (1.0)

Categorical and continuous variables are shown as frequencies (percentages) and as mean (standard deviation), respectively. * The family's financial situation was evaluated by patients themselves.

Table 2. Overall prevalence of HR-HPVs among 199 women from Kosovo according to the cytology results

HPV types	All women (N = 199)		Cytology results							
	N	Prevalence (%) (95% CI)	NILM (N = 165)		ASCUS (N = 30)		ASC-H (N = 2)		Cervical cancer (N = 2)	
			N	Prevalence (%) (95% CI)	N	Prevalence (%) (95% CI)	N	Prevalence (%) (95% CI)	N	Prevalence (%) (95% CI)
Any HR-HPV	26	13.1 (9.1–18.5)	16	9.7 (6.1–15.2)	7	23.3 (11.8–40.9)	1	50.0 (9.5–90.6)	2	100 (34.2–100)
HPV16/HPV18	10	5.0 (2.8–9.0)	5	3.0 (1.3–6.9)	2	6.7 (1.9–21.3)	1	50.0 (9.5–90.6)	2	100 (34.2–100)
HPV16	7	3.5 (1.7–7.1)	5	3.0 (1.3–6.9)	0	0 (–)	1	50.0 (9.5–90.6)	1	50.0 (9.5–90.5)
HPV18	3	1.5 (0.5–4.3)	0	0 (–)	2	6.7 (1.9–21.3)	0	0 (–)	1	50.0 (9.5–90.5)
HPV31	4	2.0 (0.8–5.1)	3	1.8 (0.6–5.2)	1	3.3 (0.6–16.7)	0	0 (–)	0	0 (–)
HPV33	1	0.5 (0.1–2.8)	1	0.6 (0.1–3.4)	0	0 (–)	0	0 (–)	0	0 (–)
HPV45	1	0.5 (0.1–2.8)	1	0.6 (0.1–3.4)	0	0 (–)	0	0 (–)	0	0 (–)
HPV51	4	2.0 (0.8–5.1)	3	1.8 (0.6–5.2)	1	3.3 (0.6–16.7)	0	0 (–)	0	0 (–)
HPV52	2	1.0 (0.3–3.6)	1	0.6 (0.1–3.4)	1	3.3 (0.6–16.7)	0	0 (–)	0	0 (–)
HPV56	1	0.5 (0.1–2.8)	1	0.6 (0.1–3.4)	0	0 (–)	0	0 (–)	0	0 (–)
HPV58	1	0.5 (0.1–2.8)	0	0 (–)	1	3.3 (0.6–16.7)	0	0 (–)	0	0 (–)
HPV66	2	1.0 (0.3–3.6)	1	0.6 (0.1–3.4)	1	3.3 (0.6–16.7)	0	0 (–)	0	0 (–)

Abbreviations — NILM (negative for intra-epithelial lesion or malignancy); ASCUS — atypical squamous cells of undetermined significance; ASC-H — atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; 95% CI — 95% confidence interval

Table 3. Distribution of HR-HPV types among 26 women infected with any of the 14 HR-HPVs according to the cytology results

HPV types	N	%	Cytology results							
			NILM		ASCUS		ASC-H		Cervical cancer	
			N	%	N	%	N	%	N	%
Any HR-HPV	26	100	16	100	7	100	1	100	2	100
HPV16/HPV18	10	38.5	5	31.3	2	28.6	1	100	2	100
HPV16	7	26.9	5	31.3	0	0	1	100	1	50.0
HPV18	3	11.5	0	0	2	28.6	0	0	1	50.0
HPV31	4	15.4	3	18.8	1	14.3	0	0	0	0
HPV33	1	3.9	1	6.3	0	0	0	0	0	0
HPV45	1	3.9	1	6.3	0	0	0	0	0	0
HPV51	4	15.9	3	18.8	1	14.3	0	0	0	0
HPV52	2	7.7	1	6.3	1	14.3	0	0	0	0
HPV56	1	3.9	1	6.3	0	0	0	0	0	0
HPV58	1	3.9	0	0	1	14.3	0	0	0	0
HPV66	2	7.7	1	6.3	1	14.3	0	0	0	0

Abbreviations — NILM (negative for intra-epithelial lesion or malignancy); ASCUS — atypical squamous cells of undetermined significance); ASC-H — atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion

regression analysis. When controlling for other variables in the model, all three independent variables were statistically significantly associated with HPV infection, with OR = 0.37 (95% CI: 0.15–0.94) and $p = 0.036$ for overall older age (over 40 years old), OR = 0.18 (95% CI: 0.06–0.50) and $p < 0.001$ for older age at sexual debut (at least 18 years old), and OR = 0.28 (95% CI: 0.09–0.92) and $p = 0.035$ for history of delivering at least one child. The results of the multiple logistic regression analysis therefore suggest that women younger than 40, who were younger than 18 at the time of first sexual intercourse and that have not yet given birth, are at greater risk for HPV infection [Fig. 1].

Table 4. Association between socio-demographic characteristics and HPV infection (results of an univariate logistic regression analysis)

	HPV-negative (n = 173)	HPV-positive (n = 26)	OR (95% CI)	P-value
(Mean) age (SD)	42.4 (9.2)	38.4 (9.9)	0.96 (0.91; 1)	0.044
< 40 years old	63 (36.4)	16 (61.5)	1	
> 40 years old	110 (63.6)	10 (38.5)	0.36 (0.15; 0.84)	0.015
Education				
Finished elementary school or less	45 (26)	6 (23.1)	1	
Finished secondary school	92 (53.2)	15 (57.7)	1.22 (0.44; 3.36)	0.697
More than secondary school	36 (20.8)	5 (19.2)	1.04 (0.29; 3.69)	0.950
Employment status				
Employed	44 (25.4)	7 (26.9)	1	
Housewife or farmer	125 (72.3)	18 (69.2)	0.91 (0.35; 2.31)	0.835
Student	4 (2.3)	1 (3.8)	1.57 (0.15; 6.18)	0.702
Marital status				
Divorced/widowed	10 (5.8)	3 (11.5)	1	
Married	155 (89.6)	21 (80.8)	0.45 (0.11; 1.77)	0.244
Single	8 (4.6)	2 (7.7)	0.83 (0.11; 6.26)	0.859
Family size				
Single	3 (1.7)	1 (3.8)	1	
Small family (parents&children)	124 (71.7)	17 (65.4)	0.41 (0.04; 4.18)	0.453
Large family (parents, children and other family members)	46 (26.6)	8 (30.8)	0.52 (0.05; 5.66)	0.593
Family income*				
Insufficient	47 (27.2)	7 (26.9)	1	
Sufficient	88 (50.9)	16 (61.5)	1.22 (0.47; 3.18)	0.683
Good	38 (22)	3 (11.5)	0.53 (0.13; 2.19)	0.380
(Mean) age (SD) at first sexual intercourse	21.9 (4.2)	20.8 (5.4)	0.94 (0.84; 1.04)	0.230
< 18 years old	22 (12.7)	9 (34.6)	1	
> 18 years old	151 (87.3)	17 (65.4)	0.28 (0.11; 0.69)	0.006
Delivery				
No	15 (8.7)	6 (23.1)	1	
Yes	158 (91.3)	20 (76.9)	0.32 (0.11; 0.91)	0.026
Pregnancy termination				
No	81 (46.8)	18 (69.2)	1	
Yes	92 (53.2)	8 (30.8)	0.39 (0.16; 0.95)	0.033
Menstrual cycle				
Irregular	33 (19.1)	7 (26.9)	1	
Regular	108 (62.4)	18 (69.2)	0.79 (0.30; 2.04)	0.620
Postmenopausal	32 (18.5)	1 (3.8)	0.15 (0.02; 1.27)	0.081
Contraception				
No	145 (83.8)	21 (80.8)	1	
Yes	28 (16.2)	5 (19.2)	1.23 (0.43; 3.54)	0.697
Smoking				
No	131 (75.7)	18 (69.2)	1	
Yes	42 (24.3)	8 (30.8)	1.39 (0.56; 3.42)	0.477

Categorical and continuous variables are shown as frequencies (percentages) and as mean (standard deviation), respectively. * The family's financial situation was evaluated by patients themselves. Abbreviations — OR (odds ratio); 95% CI — 95% confidence interval

Association between sociodemographic characteristics and HR-HPV types

As shown in Table 5, the results of the likelihood ratio test suggested that women infected with HPV16 were older in comparison to women infected with other HR-HPV types ($p = 0.037$). In addition, all women infected with HPV16 have given birth to at least one child, whereas only 68.4% of women infected with other HR-HPVs had biological children ($p = 0.036$). Other characteristics were not statistically significantly associated with specific HR-HPV types.

DISCUSSION

Infections with over 40 HPV types from the clinically most important *Alpha*-PV genus are considered to be the most frequently sexually transmitted infections in both genders. In addition, worldwide, up to 4.5% of incident cancer cases, including cervical, anogenital, and head and neck cancers, are etiologically associated with HPV infection. Moreover, cervical cancer accounts for 83% of all HPV-related cancers, with the majority occurring in women originating from less-developed countries [4].

In the female population from Kosovo, the crude overall prevalence of infection with any of the 14 HR-HPVs was estimated at 13.1%. Interestingly, in our study population the overall HR-HPV prevalence in NILM was lower than in studies on a total of 10.744 eligible women from other countries in central and eastern Europe (9.7%; 95% CI: 6.1–15.2% vs. 18.0%; 95% CI: 17.0–19.0%, respectively) [15–23], with reported high incidence rates of cervical cancer and related mortality rates [1]. Our results were concordant with those obtained in the largest study in the region to date, performed on 4.199 Slovenian women (HR-HPV prevalence of 10.7%; cervical cancer incidence rate per 100,000: 13.4; mortality rate due to cervical cancer per 100,000: 6.1) [1, 14], as well as with global data (HR-HPV prevalence of 10.4%; cervical cancer incidence rate per 100,000: 15.1; mortality rate due to cervical cancer per 100,000: 7.6) [1, 5].

Three prophylactic HPV vaccines are commercially available: a bivalent HPV16/18 vaccine (Cervarix; GlaxoSmithKline Biologicals, Belgium), a quadrivalent HPV6/11/16/18 vaccine (Gardasil/Silgard; Merck & Co., USA / Sanofi Pasteur MSD, France), and a nonavalent HPV6/11/16/18/31/33/45/52/58 vaccine (Gardasil9, Merck & Co.). After the first 10 years of routine use of HPV vaccines, comprehensive clinical trials and real-life data have confirmed the vaccines' safety and effectiveness.

Even though the introduction of HPV vaccination has the potential to substantially reduce the incidence of cervical cancer cases, the full effect on women of all ages will be detectable no sooner than after 30 years. Thus well-organized cervical cancer screening programs will still play a crucial role in the prevention of cervical cancer and bridging the gap until the full effect of HPV vaccines can be observed [24]. Vaccarella et al. estimated recently that, with the implementation of well-organized cervical cancer screening by 2017 in six Baltic, central European, and eastern European countries (Estonia, Lithuania, Latvia, Belarus, Bulgaria, and Russia), in which cervical cancer screening is absent or, at best, opportunistic with low coverage and quality, 180,000 new cervical cancer cases could be prevented from 2017 to 2040 [24]. In comparison to cytology-based screening, HPV-based screening is more effective and efficient for the prevention of invasive cervical cancer. Therefore, HPV testing is an invaluable part of guidelines for cervical cancer screening, triage, and follow-up after treatment [24]. Even though more than 190 distinct HPV tests and at least 127 test variants were commercially available in 2015, only a fraction of HPV tests fulfill the criteria for use in primary cervical cancer screening, with RealTi*m*e, used in this study, being one of them [25]. The World Health Organization suggests that, in countries without established cytology-based cervical cancer screening, HPV-based screening programs, in combination with the implementation of HPV vaccination, could further accelerate screening benefits and reduce the burden of cervical cancer [24].

Despite the fact that a comprehensive strategy, including HPV vaccination and HPV-based cervical cancer screening, has been demonstrated to be cost-effective in nearly all countries [26], progress toward prevention is often hindered due to relatively low access to vaccines [27] and limited use of cervical cancer screening [28], especially in low-income countries.

We hope that the results of this study will also persuade the authorities to implement nationwide cervical cancer screening and HPV vaccination programs in Kosovo.

Even though multiple HPV infections are not uncommon in cervical specimens, each HPV type is associated with a biologically separate and independent cervical lesion, as observed using laser capture micro-dissection (LCM) with HPV PCR typing (LCM-PCR) [29]. In our study, multiple HPV types were detected in five cervical smears (HPV16+HPV84, HPV66+HPV67, HPV51+53, HPV51+62, and HPV6+58+73). Although the cycle threshold values obtained using the RealTi*m*e were higher for HR-HPV types in comparison to other HPVs, suggesting the predominance of HR-HPVs, it is impossible to speculate on the etiological associations because the LCM-PCR technique cannot be used on smear samples.

Because persistent infection with HR-HPVs is a necessary, but non-sufficient, etiological factor of cervical cancer, several contributory factors have been described previously [3, 4, 30]. In this study, the results of the univariate and multiple logistic regression analyses suggested that women over 40 (univariate logistic regression: $p = 0.015$, OR = 0.36;

Table 5. Association between socio-demographic characteristics and HR-HPV types (results of likelihood ratio test)

	Other HPV type (n = 19)	HPV16 (n = 7)	P-value
Age			
< 40 years old	14 (73.7)	2 (28.6)	0.037
> 40 years old	5 (26.3)	5 (71.4)	
Education			
Finished elementary school or less	6 (31.6)	0 (0)	0.107
Finished secondary school	10 (52.6)	5 (71.4)	
More than secondary school	3 (15.8)	2 (28.6)	
Employment status			
Eemployed	5 (26.3)	2 (28.6)	0.725
Housewife or farmer	13 (68.4)	5 (71.4)	
Student	1 (5.3)	0 (0)	
Marital status			
Married	15 (78.9)	6 (85.7)	0.303
Divorced/widowed	3 (15.8)	0 (0)	
Single	1 (5.3)	1 (14.3)	
Family size			
Single	1 (5.3)	0 (0)	0.706
Small (parents&children)	12 (63.2)	5 (71.4)	
Large (parents, children and other family members)	6 (31.6)	2 (28.6)	
Family income*			
Insufficient	6 (31.6)	1 (14.3)	0.652
Sufficient	11 (57.9)	5 (71.4)	
Good	2 (10.5)	1 (14.3)	
Age at first sexual intercourse			
< 18	7 (36.8)	2 (28.6)	0.691
> 18	12 (63.2)	5 (71.4)	
Delivery			
Yes	13 (68.4)	7 (100)	0.036
No	6 (31.6)	0 (0)	
Pregnancy termination			
Yes	6 (31.6)	2 (28.6)	0.882
No	13 (68.4)	5 (71.4)	
Menstrual cycle			
Regular	13 (68.4)	5 (71.4)	0.725
Irregular	5 (26.3)	2 (28.6)	
Postmenopausal	1 (5.3)	0 (0)	
Contraception			
Yes	4 (21.1)	1 (14.3)	0.691
No	15 (78.9)	6 (85.7)	
Smoking			
Yes	6 (31.6)	2 (28.6)	0.882
No	13 (68.4)	5 (71.4)	

* The family's financial situation was evaluated by patients themselves.

multiple logistic regression: $p = 0.036$, OR = 0.37), who were older than 18 at sexual debut (univariate logistic regression: $p = 0.006$, OR = 0.28; multiple logistic regression: $p < 0.001$, OR = 0.18), and those with a history of delivering at least one child (univariate logistic regression: $p = 0.026$, OR = 0.32; multiple logistic regression: $p = 0.035$, OR = 0.28) were at lower risk for HPV infection. The results of the univariate logistic regression analysis additionally suggested that women with a history of pregnancy termination also have lower odds ($p = 0.033$, OR = 0.39) for HPV infection. Our results are concordant with global data (reviewed in 30), reporting that the incidence/prevalence of HPV infection peaks at a younger age (below 25–35 years), soon after the start of sexual activity, and declines with age due to the spontaneous clearance of HPV infections. Similarly as in our study, associations between cervical cancer, cervical intraepithelial lesions, and increasing number of pregnancies and parity were also found in previous case-control studies (reviewed in 30). Interestingly, according to the likelihood ratio test, in comparison to women infected with other HR-HPV types, women infected with HPV16 were older ($p = 0.037$) and have given birth to at least one child ($p = 0.036$).

CONCLUSIONS

In conclusion, this study is the first study reporting the HR-HPV prevalence and distribution of HPV types in Kosovo. Because more than 70% of cervical precancerous lesions identified in the study could have been prevented using nationwide HPV-based cervical cancer screening and HPV vaccination programs, it is of outmost importance to implement both programs in the national health care system in Kosovo as soon as possible.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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