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Short communication

Pre-vaccination prevalence and distribution of high-risk human papillomavirus (HPV) types in Slovenian women: A cervical cancer screening based study

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ABSTRACT

To estimate the pre-vaccination prevalence of cervical infections with 14 high-risk human papillomavirus (hr-HPV) types among 20–64 years old Slovenian women screened for cervical cancer in 2010, we consecutively enrolled 4431 women in 16 outpatient gynaecology services. All were screened with Digene Hybrid Capture 2 HPV DNA Test and Abbott Real Time High Risk HPV Test and all positive specimens genotyped. Prevalence of cervical infection with any hr-HPV type examined was 12.9% with HPV16 3.5% and with HPV18 1.0%. Age specific prevalence estimates were the highest among 20–24 years old women and decreased with age. HPV16 prevalence was lowest among women without evidence of cervical disease and increased with the severity to 41.9% in women with high grade squamous intraepithelial lesion. Our results provide baseline data for monitoring the impact of Slovenian HPV vaccination program and development of future cervical cancer screening strategies in cohorts eligible for free HPV vaccination.

1. Introduction

Infections with over 40 human papillomavirus (HPV) are most common sexually transmitted infections [1]. Persistent infection with at least one of 12 sexually transmitted HPV types (traditionally designated as "high-risk" types) is necessary but non-sufficient cause of virtually all cervical cancers and a substantial proportion of vaginal, anal, penile, vulvar and oropharyngeal cancers [2–5]. Cervical cancer is most frequently caused by HPV16, followed by HPV18, together causing approximately 71% of the estimated global burden [6]. The relative importance of high-risk HPV (hr-HPV) types varies among different populations [7,8]. Information on hr-HPV infection prevalence and hr-HPV type distribution from large population based studies has not been available from most countries of Central and Eastern Europe [9].

Two vaccines against HPV infection have been licensed in European Union (EU), the quadrivalent vaccine containing L1 virus-like particles of HPV types 6, 11, 16 and 18, preventing premalignant genital lesions (cervical, vulvar and vaginal), cervical cancer and external genital warts [10,11] and bivalent vaccine containing L1 virus-like particles of HPV types 16 and 18, preventing premalignant cervical lesions and cervical cancer [12,13].

In Slovenia, self-paid vaccination against HPV with quadrivalent vaccine became available by the end of 2006 and with bivalent vaccine in 2007. In 2009, free of charge vaccination of 11–12 years old girls with quadrivalent vaccine was introduced into the Slovenian National Vaccination Program in the absence of reliable data on the prevalence of HPV infection among Slovenian women.

Among Slovenian women screened for cervical cancer, we estimated the pre-vaccination prevalence of cervical infections with 14 HPV types (overall, according to age and stage of cervical disease) and among those infected with any hr-HPV type, we described the type-specific distribution according to different stages of cervical disease.

2. Methods

2.1. Study population

Women 20–64 years old eligible for a preventive cytological examination of the cervical smear according to the criteria of the National Cervical Cancer Screening Program (NCCSP) [14] were consecutively enrolled within a convenience sample of 16 outpatient gynaecology services all over the country between December 2009 and August 2010. Exclusion criteria were: attendance after an atypical/abnormal cytology result, history of cervical intraepithelial neoplasia of any grade, treatment for cervical disease in the preceding year, hysterectomy, and menstruating or pregnancy at presentation.

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Table 1
Prevalence of infection with high-risk HPV types, overall and according to cytology result among 4431 women screened for cervical cancer, Slovenia, 2010.

HPV types	All women (*N* = 4431)			Cytology result											
	N	Prevalence (%)	95% CI	NILM (*N* = 4199)			Atypical cells[a] (*N* = 120)			LSIL (*N* = 69)			HSIL (*N* = 43)		
				N	Prevalence (%)	95% CI	*N*	Prevalence (%)	95% CI	*N*	Prevalence (%)	95% CI	*N*	Prevalence (%)	95% CI
Any hr-HPV	574	12.9	12.0–13.9	451	10.7	9.8–11.7	37	30.8	22.4–39.2	50	72.5	61.6–83.3	36	83.7	72.2–95.2
Multiple hr-HPV[b]	158	3.6	3.0–4.1	113	2.7	2.2–3.2	16	13.3	7.2–19.5	23	33.3	21.9–44.7	6	13.9	3.2–24.7
HPV16 or/and 18[c]	195	4.4	3.8–5.0	137	3.3	2.7–3.8	15	12.5	6.5–18.5	22	31.9	20.6–43.2	21	48.8	33.3–64.4
HPV16	155	3.5	2.9–4.0	105	2.5	2.0–3.0	14	11.7	5.8–17.5	18	26.1	15.5–36.7	18	41.9	26.5–57.2
HPV18	46	1.0	0.7–1.3	37	0.9	0.6–1.2	1	0.8	0.0–2.4	5	7.2	1.0–13.5	3	7.0	0.0–14.9
HPV31	114	2.6	2.1–3.0	92	2.2	1.7–2.6	11	9.0	3.9–14.4	5	7.2	1.0–13.5	6	13.9	3.2–24.7
HPV33	32	0.7	0.5–1.0	24	0.6	0.3–0.8	1	0.8	0.0–2.4	2	2.9	0.0–6.9	5	11.6	1.6–21.6
HPV35	9	0.2	0.1–0.3	8	0.2	0.1–0.3	0	0	–	0	0	–	1	2.3	0.0–7.0
HPV39	50	1.1	0.8–1.4	46	1.1	0.8–1.4	2	1.7	0.0–4.0	2	2.9	0.0–6.9	0	0	–
HPV45	42	0.9	0.7–1.2	28	0.7	0.4–0.9	4	3.3	0.1–6.6	7	10.1	2.8–17.4	3	7.0	0.0–14.9
HPV51	81	1.8	1.4–2.2	68	1.6	1.2–2.0	5	4.2	0.5–7.8	6	8.7	1.9–15.5	2	4.6	0.0–11.2
HPV52	78	1.8	1.4–2.2	61	1.4	1.1–1.8	6	5.0	1.0–8.9	8	11.6	3.8–19.3	3	7.0	0.0–14.9
HPV56	31	0.7	0.4–0.9	16	0.4	0.2–0.6	6	5.0	1.0–8.9	7	10.1	2.8–17.4	2	4.6	0.0–11.2
HPV58	29	0.6	0.4–0.9	23	0.5	0.3–0.8	2	1.7	0.0–4.0	3	4.3	0.0–9.3	1	2.3	0.0–7.0
HPV59	48	1.1	0.8–1.4	34	0.8	0.5–1.1	5	4.2	0.5–7.8	9	13.0	4.9–21.2	0	0	–
HPV66	47	1.0	0.7–1.3	33	0.8	0.5–1.0	5	4.2	0.5–7.8	9	13.0	4.9–21.2	0	0	–
HPV68	25	0.6	0.3–0.8	22	0.5	0.3–0.7	1	0.8	0.0–2.4	2	2.9	0.0–6.9	0	0	–

NILM: negative for intraepithelial lesion and malignancy; LSIL: low-grade squamous intraepithelial lesions; HSIL: high-grade squamous intraepithelial lesions; hr-HPV: high-risk HPV types; *N*: number of women; CI: confidence interval.

[a] Atypical cells: atypical squamous cells of undetermined significance (ASC-US) (109 women), atypical squamous cells, cannot exclude HSIL (ASC-H) (8 women) and atypical glandular cells (3 women).

[b] Women with multiple high-risk HPV types infection were also counted as positive for the particular individual high-risk HPV infection.

[c] Women with infection with only high-risk HPV16 or only high-risk HPV18 or infection with both.

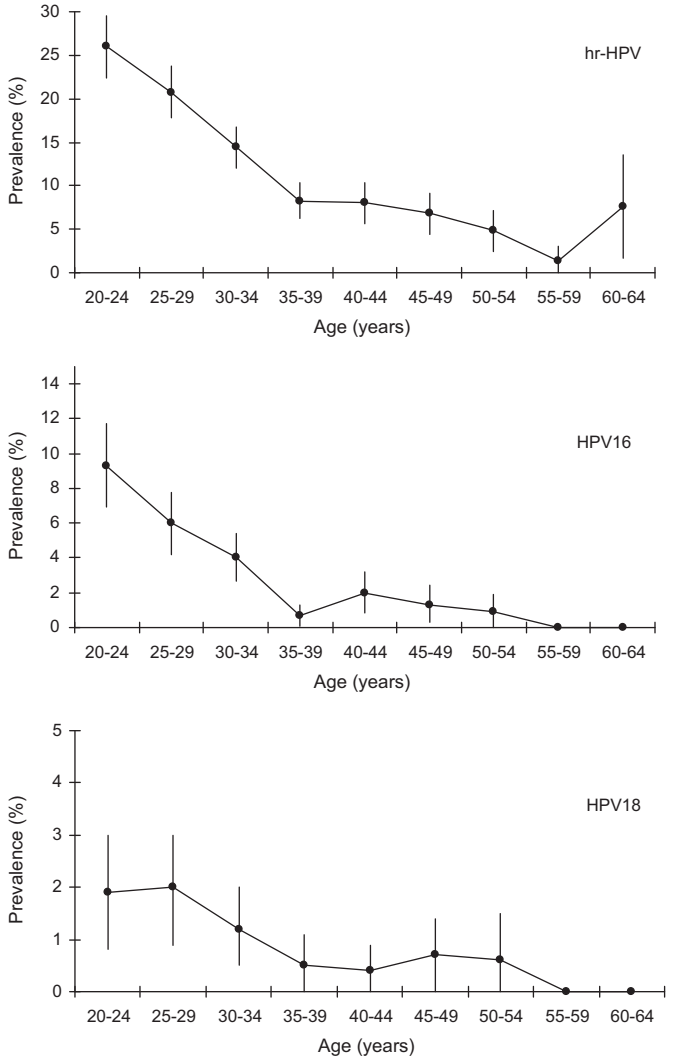

Fig. 1. Prevalence of infection with high-risk HPV types with 95% confidence intervals according to age among 4431 women screened for cervical cancer, Slovenia, 2010.

2.2. Specimens and data collection

Gynaecological examination including visualization of cervix and collection of cervical smear specimen for cytological examination was performed according to the NCCSP guidelines [15]. Specimens were examined under routine screening conditions in certified cytological laboratories normally used by participating sites by cytologists who were not aware of the results of HPV testing. Women were called for colposcopy using a cytology threshold of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion/atypical glandular cells or worse [15]. Irrespective of cytology result, women positive for HPV16 or HPV18 were also invited for colposcopy. A second specimen was obtained for HPV DNA testing and placed into ThinPrep PreservCyt Solution (Hologic, Marlborough, MA), as described previously [16]. Participants anonymously completed a self-administered questionnaire that included a question on vaccination against HPV.

2.3. HPV testing and genotyping

The complete protocol of HPV testing, genotyping and discordant analysis used in this study is described in details elsewhere [16]. Briefly, all specimens were first tested in parallel with Hybrid Capture 2 HPV DNA Test (hc2) (Qiagen, Hilden, Germany) and RealTime High Risk HPV Test (RealTime) (Abbott, Wiesbaden, Germany). Hc2 has been used in the majority of key trials that have proved the clinical value of hr-HPV testing [17]. RealTime is a new-generation real-time PCR-based assay, which detects a pool of 12 carcinogenic HPV genotypes in aggregate (HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68), with concurrent, separate detection of HPV16 and HPV18 [16,18]. All specimens with RealTime/hc2 concordant positive results and RealTime/hc2 discordant results were tested using the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Branchburg, NJ), and if necessary, with an HPV52 type-specific real-time PCR assay, INNO-LiPA HPV Genotyping *Extra* Test (Innogenetics, Gent, Belgium), and an in-house GP5+/GP6+ PCR assay targeting a 150-bp fragment in HPV L1 gene with additional HPV68 specific primers, as described previously [16]. The analytical reliability of the applied genotyping strategy was verified using the

Table 2
Distribution of infections with high-risk HPV types among 574 women infected with any of 14 high-risk HPV types according to cytology result among 4431 women screened for cervical cancer, Slovenia, 2010.

HPV types	*N*	%	Cytology result							
			NILM		Atypical cells[a]		LSIL		HSIL	
			N	%	*N*	%	*N*	%	*N*	%
Any hr-HPV	574	100.0	451	100.0	37	100.0	50	100.0	36	100.0
HPV16 or/and 18	195	34.0	137	30.4	15	40.5	22	44.0	21	58.3
HPV16	155	27.0	105	23.3	14	37.8	18	36.0	18	50.0
HPV18	46	7.8	37	8.2	1	2.7	5	10.0	3	8.3
HPV31	114	19.9	92	20.4	11	29.7	5	10.0	6	16.7
HPV33	32	5.6	24	5.3	1	2.7	2	4.0	5	13.9
HPV35	9	1.6	8	1.8	0	0	0	0	1	2.8
HPV39	50	8.7	46	10.2	2	5.4	2	4.0	0	0
HPV45	42	7.3	28	6.2	4	10.8	7	14.0	3	8.3
HPV51	81	14.1	68	15.1	5	13.5	6	12.0	2	5.5
HPV52	78	13.6	61	13.5	6	16.2	8	16.0	3	8.3
HPV56	31	5.4	16	3.5	6	16.2	7	14.0	2	5.5
HPV58	29	5.0	23	5.1	2	5.4	3	6.0	1	2.8
HPV59	48	8.4	34	7.5	5	13.5	9	18.0	0	0
HPV66	47	8.2	33	7.3	5	13.5	9	18.0	0	0
HPV68	25	4.4	22	4.9	1	2.7	2	4.0	0	0

NILM: negative for intraepithelial lesion and malignancy; LSIL: low-grade squamous intraepithelial lesions; HSIL: high-grade squamous intraepithelial lesions; hr-HPV: high-risk HPV types; *N*: number of women.

[a] Atypical cells: atypical squamous cells of undetermined significance (ASC-US) (109 women), atypical squamous cells cannot exclude HSIL (ASC-H) (8 women) and atypical glandular cells (3 women).

HPV DNA Proficiency 2010 Panel prepared by the World Health Organization HPV Laboratory Network – LabNet [19], as described previously [16].

2.4. Statistical analysis

Statistical analyses were performed using the STATA package version 10.0 (Stata Statistical Software: release 10.0 College Station, TX, Stata Corporation). We estimated the prevalence of infections with any and individual 14 hr-HPV types with 95% confidence intervals (CI). Using the direct method of standardization, we computed age and cytology-standardized prevalence of infection with any hr-HPV type using as the standard the population of Slovenian women screened within the NCCSP in 2009, and age-standardized prevalence using as the standard the Slovenian female population as recorded in the Central Population Registry (CPR) in 2010. Chi-square test for linear trend was used 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 and to assess the association between the prevalence of infection with any hr-HPV type and severity of cervical disease. Level of statistical significance was set at $p < 0.05$.

2.5. Ethics

The study has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) and has been approved by the Medical Ethics Committee of the Republic of Slovenia (consent number: 83/11/09).

3. Results

From a total of 4514 participants (98.1% response), 83 were excluded from analysis (not 20–64 years old, specimen spill out during transport/missing, invalid results of internal amplification control, inconclusive result of HPV genotyping, missing cytology, vaccinated against HPV).

The crude overall prevalence of infection with any hr-HPV type was 12.9% (95% CI: 12.0–13.9%). Corresponding NCCSP population in 2009 age-standardized estimate was 11.8% and cytology standardized estimate 12.9%. Corresponding age-standardized estimate for CPR population 20–64 years old was 10.6% and for 30–64 years old 7.3%.

Overall prevalence of infections with hr-HPV types and prevalence of infections with HPV16 and HPV18 were the highest among 20–24 years old women and decreased with age (all $p < 0.001$) (Fig. 1).

Prevalence of infection with hr-HPV types, overall and according to cytology result is shown in Table 1. Any hr-HPV type infection prevalence was the lowest among participants negative for intraepithelial lesion and malignancy (NILM) and increased with the severity of the cervical disease ($p < 0.001$). Irrespective of cytology result, the most prevalent individual hr-HPV type infection was infection with HPV16.

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 2.

4. Discussion

We have estimated the pre-vaccination prevalence of cervical infections with selected 14 hr-HPV types among 20–64 years old Slovenian women eligible for cervical cancer screening. Similar to other European countries, cervical infection with HPV16, the HPV type with the strongest oncogenic potential, was most common overall and among women with cervical disease [9].

Although probability sampling was not used, our study population had similar characteristics to the total NCCSP population with respect to age and cervical pathology (data not shown) and we believe that our estimates reflect fairly well the true prevalence of hr-HPV cervical infection among Slovenian women screened for cervical cancer. Since, the NCCSP has a fairly good coverage (in last 3 years more than 70% of targeted women had at least one smear [20]) we assume that our estimates also fairly well reflect the prevalence of cervical infection with hr-HPV among 20–64 years old Slovenian women eligible for screening.

Our results provide baseline data for monitoring the impact of Slovenian HPV vaccination program (including hr-HPV type distribution change), and development of future cervical cancer screening strategies in cohorts eligible for free HPV vaccination. The data could also be used for health economic and modelling research.

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