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Human papillomavirus infection in women with and without cervical cancer in Warsaw, Poland

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ABSTRACT

Cervical cancer incidence and mortality in Poland is among the highest in Europe. To investigate infection with different human papillomaviruses (HPV) in Warsaw, Poland, we obtained cervical cell specimens from 834 women aged 18–59 years from the general population, and 88 cervical cancers. DNA of 44 HPV types was detected using a GP5+/6+-based PCR assay. HPV prevalence was 16.6% in the general female population, being highest (24.2%) in women aged 25–34 years, notably among unmarried women (37.3%). HPV prevalence fell to 8.6% at ages 55–59. High-risk HPV prevalence was 11.3%, with HPV16 being the most common type (3.7%). All but one cervical cancer were high-risk HPV-positive, although the importance of HPV16 (73%) was much greater, and multiple infections fewer (1%), than among HPV-positive women in the general female population. In summary, we report a relatively high burden of HPV infection in Warsaw, Poland, where 79% of cervical cancers are theoretically preventable by HPV16/18 vaccines.

1. Introduction

The incidence of cervical cancer in Eastern Europe is approximately three-fold higher than in Western European countries, with particularly elevated rates reported in Poland (14.4/100,000 women in Warsaw, 19.6/100,000 women in Cracow).[1] The disparity between Eastern and Western Europe is also reflected in cervical cancer mortality rates.[2] Population-based screening programmes in Poland are few and restricted to small areas, but at least in urban districts, opportunistic cytological screening is widespread.[3]

The establishment of genital infection with certain types of human papillomavirus (HPV) as the central cause of cervical cancer has prompted a shift in the planning of primary and secondary prevention towards HPV test-based screening[4] and vaccination.[5]

One of the essential pieces of epidemiological data required to introduce and predict the impact of HPV test-based screening or vaccination in a given country is population-based data on age- and type-specific patterns of HPV prevalence, which is known to vary substantially across different populations.[6,7] In addition, corresponding information on women with cervical cancer is important to predict the theoretical fraction of cervical cancer preventable by current HPV16/18 vaccines.

Population-based studies on the prevalence of HPV in Eastern European countries remain limited.[8,9] Therefore, the International Agency for Research on Cancer (IARC), in

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collaboration with The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland, have carried out an HPV survey on a representative sample of women from Warsaw, and on a corresponding sample of locally-diagnosed cervical cancer cases.

2. Materials and methods

2.1. Ethical approval

All participants, whether from the general female population or those with cervical cancer, signed informed consent forms according to the recommendations of the IARC and the Maria Sklodowska-Curie Memorial Institute and Cancer Center ethical review committees, which both approved the study.

2.2. General female population

The survey of HPV infection in the general female population was carried out between March and May 2006 in Warsaw, Poland. A population-based cervical screening programme, including an active call of women aged 25–59 years, was already present in two of 18 districts of Warsaw (Ursynow and Mokotow), and therefore two different recruitment methods were used.

The first method, used in districts where screening programmes did not exist (~440,000 eligible women), consisted of a random selection of an age-stratified sample of women aged 18–59 years from the Polish Electronic Registry. This country-wide registry is a compulsory and regularly updated government-run system, registering all individuals at birth. For each 5-year age group in the 25–59-year range, 130 women were selected and invited to join the present study. Among a total of 910 invitees, 424 (47%) chose to participate. On account of the importance of information on young women,[7] women in the 18–24-year age group were over-sampled and, out of 400 women invited, 163 (41%) agreed to participate. All potential participants were invited by personal letter to the Department of Cervical Cancer Screening at the Maria Sklodowska-Curie Memorial Institute and Cancer Center.

Conversely, in the Ursynow and Mokotow Districts, the recruitment method took advantage of invitations sent by two long-established cervical cancer screening programmes in these areas.[3] All female residents aged 25–59 years (approximately 36,000 in Ursynow and 50,000 in Mokotow) are invited every 3 years to the Department of Cervical Cancer Screening at the Maria Sklodowska-Curie Memorial Institute and Cancer Center for a Pap smear. Participation in the screening programme is estimated to be about 58% for each 3-year cycle.[3] Approximately 40 screening programme attendees from each 5-year age group in the 25–59-year range were invited to participate, and among 294 invited women, only 14 refused. An additional group of women who lived in the Ursynow and Mokotow Districts, aged 18–24 years but too young to be invited to join the regular screening programme, were also invited and 45 agreed to participate.

A total of 912 women (325 from Ursynow/Mokotow and 587 from the rest of Warsaw) thus agreed to participate in the population-based study. Women enrolled from Ursynow and Mokotow Districts did not differ significantly from the wider sample of Warsaw residents in respect to education, marital status, lifetime number of sexual partners, nor HPV prevalence.

For both recruitment methods, exclusion criteria were previous hysterectomy, pregnancy beyond the 34th week at time of recruitment, and mental or physical incompetence.

A structured questionnaire including information on socio-demographic characteristics, sexual behaviour of the women and of their partners, reproductive factors, use of contraceptive methods and smoking habits was administered to all study participants.

A total of 897 women in the population-based sample accepted to undergo a pelvic examination performed by a gynaecologist and to give 10 ml of blood. After the preparation of a conventional Pap smear, a sample of exfoliated cervical cells from the endocervix and ectocervix was collected with a cytobrush (Cervexbrush, Rovers Medical Devices B.V., The Netherlands). The brush containing cellular material was then placed in a vial containing PreserveCyt medium (Cytyc, Boxbourough, MA, USA) and stored at +4 °C until shipment.

Pap smears were read at the Cytological Department of the Maria Sklodowska-Curie Memorial Institute and Cancer Center, Warsaw, Poland. Specimens were reviewed by experienced cytologists and additionally confirmed by a pathologist, according to the 2001 Bethesda System. Women with abnormal cervical findings were referred for further diagnostic procedures according to standard local protocols.

2.3. Women with invasive cervical cancer

Cervical cancer cases were identified at the Maria Sklodowska-Curie Memorial Institute and Cancer Center. Out of 121 women diagnosed with cervical cancer at the Cancer Center between March 2006 and February 2007, the collection of a tumour biopsy for the purposes of the study was possible for 107 (88%). Biopsies were formalin-fixed and embedded in paraffin, before being sectioned for HPV testing using a 'sandwich' approach, whereby inner tumour sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue. Biopsies without any histological evidence of tumour tissue ($n = 15$) or suspected to be of endometrial origin ($n = 4$) were excluded from all analyses.

2.4. HPV detection

HPV testing was performed on exfoliated cervical cells and cervical cancer biopsies in the Department of Pathology at the Vrije Universiteit Medical Center, Amsterdam, The Netherlands. DNA was extracted from the PreserveCyt sample using a High Pure PCR product Purification Kit according to the manufacturer's instructions (Roche Applied Science, Mannheim, Germany). For cervical cancer biopsies, one or more 5 μM sections representing approximately 1 cm^2 of tissue, were digested with Proteinase K in the presence of 0.45% Tween 20.[10] Following heat inactivation of the Proteinase K, this crude extract was used for PCR.

β-globin PCR analysis was performed firstly to confirm the presence of human DNA in all specimens.[11] In the event that crude biopsy extracts were β-globin negative, samples were subjected to DNA extraction using a High Pure PCR product

Purification Kit, and resulting DNA isolates were re-checked for β-globin. The overall presence of HPV DNA was determined by performing a general GP5+/6+ primer-mediated PCR, which permits the detection of a broad spectrum of genital HPV types at the subpicogram level.[12] HPV positivity was assessed by hybridisation of PCR products in an enzyme immunoassay using two HPV oligoprobe cocktails that, together, detect the following 44 HPV types: HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4 subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), cand85, 86, cand89 (equivalent to CP6108) and JC9710. Subsequent HPV typing was performed by reverse-line blot hybridisation of PCR products, as described previously.[13]

HPV types considered high-risk for this analysis included HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82.[14]

2.5. *Statistical analysis*

Odds ratios (ORs) for HPV positivity and corresponding 95% confidence intervals (CIs) were calculated using unconditional logistic regression. Two models were used in the population-based study: 1) adjusted for age (18–24, 25–34, 35–44, 45–54, 55–59 years); and 2) adjusted for age and lifetime number of sexual partners (1, 2–3, 4–5, ⩾6). Tests for linear trend of ORs were done giving an increasing score for each level of the categorised variable and fitting them into the model as continuous variables. Prevalence ratios (PRs) for type-specific HPV positivity and corresponding 95% CIs were used to compare the relative frequency of the most common HPV types in cervical cancer with that among HPV-positive women with normal cytology.

3. Results

3.1. *General female population*

Of 897 women who provided cervical cell samples, 49 had inadequate HPV results (β-globin negative), four had inadequate cytology, and 10 had undergone hysterectomy, leaving 834 women with valid cytology and HPV results. Among them, 35 (4.2%) had abnormal cytological findings, including 10 (1.2%) women with atypical squamous cells of undetermined significance, 22 (2.6%) with low-grade squamous intraepithelial lesions and three (0.4%) with high-grade squamous intraepithelial lesions. Final histological diagnosis was obtained for 30 women with abnormal cytology, among whom six cervical intraepithelial neoplasia grade 3/*carcinoma in situ* were diagnosed.

Overall HPV prevalence was 16.6% (65.7% and 14.4% among women with and without cervical abnormalities, respectively, Table 1). The corresponding prevalence age-standardised to the world population was 17.8% (95% CI: 15.0–20.5). In total, 97 (10.8%) women had single-type and 41 (4.6%) had multiple-type infections. High-risk HPV types were detected slightly more frequently (11.3%) than low-risk types (9.8%). The most common high-risk types among women without cervical abnormalities were HPV16 (2.8%), 56 (1.8%), 45 (1.5%), 31 (1.3%) and 52 (1.3%). HPV16 was also the most common type among women with cervical abnormalities (25.7%), of whom 48.6% were positive for high-risk types.

Fig. 1 shows the prevalence of HPV (any type, HPV16 and/or 18, high- and low-risk types, separately) by age group. Overall HPV prevalence was 21.6% among women younger than 25 years, and reached 24.2% in the 25–34-year age group. Thereafter, it steadily decreased with age down to 8.6% at age 55–59 years. Age-specific patterns were similar for the prevalence of high-risk types and HPV16 and/or 18 (Fig. 1). When women below the age of 25 years were further subdivided into two age groups, overall HPV prevalence increased between 18–22 (18.2%) and 23–24 years (26.4%) (data not shown).

Age-specific HPV prevalence is compared by marital status in Fig. 2. Among women younger than 25 years, HPV prevalence was similar between unmarried (including single, widowed or separated) (22.0%) and married women (17.2%). In the age group 25–34 years, however, HPV prevalence was significantly higher (37.3%) among unmarried than married women (16.8%). At older ages, HPV prevalence remained higher in unmarried than married women, although 95% CIs overlapped (Fig. 2).

Table 2 shows the relationship between HPV positivity and various characteristics of study participants. The strong decreases in HPV positivity seen with increasing age were confirmed to be significant, even after adjustment for lifetime number of sexual partners (OR for 55–59 versus 18–24 years = 0.2; 95% CI: 0.1–0.6). Significant associations of HPV positivity with single (OR = 2.1; 95% CI: 1.3–3.4) or widowed/separated women (OR = 2.3; 95% CI: 1.2–4.3) compared to married women, were attenuated, but remained after adjustment for lifetime number of sexual partners. HPV positivity increased sharply with an increasing lifetime number of sexual partners and the OR for six or more versus one lifetime sexual partner was 8.3 (95% CI: 4.5–15.6). An inverse relationship was found between HPV positivity and age at sexual debut with the model adjusted for age only, but the association was no longer statistically significant after adjustment for lifetime number of sexual partners. Conversely, women who reported that their current partner had had extramarital relationships had a significantly increased risk (OR = 3.3; 95% CI: 1.6–6.8) of being HPV-positive, even after adjustment for a woman's lifetime number of sexual partners. Parity was inversely related to HPV positivity in the age-adjusted model, but not after adjustment for lifetime number of sexual partners (Table 2).

No significant association with HPV positivity was found for education level or smoking status (data not shown). Neither smoking intensity (OR for ⩾15 cig/day = 1.1; 95% CI: 0.3–3.3) nor duration (OR for ⩾20 years = 0.8; 95% CI: 0.3–1.6) were related to HPV positivity. Furthermore, no significant associations with HPV positivity were found for having ever had a Pap smear (reported by 93% of women), spontaneous abortion (13%), voluntary abortion (13%), oral contraceptive use (46%), intrauterine device use (14%), condom use (59%), or menopause (17%) (data not shown).

3.2. *Women with invasive cervical cancer*

Type-specific HPV prevalence is reported in Table 3 for 88 cases of invasive cervical cancer with valid HPV and histological test results, including 84 squamous cell carcinomas and

Table 1 – Prevalence of human papillomavirus (HPV) types among 834 women from the general population, by cytological findings (Warsaw, Poland, 2006)

HPV type	Cytology								
	Normal			Abnormal			Total		
	Single	Multiple	Total (%)	Single	Multiple	Total (%)	Single	Multiple	Total (%)
Negative			684 (85.6)			12 (34.3)	–	–	696 (83.4)
Positive	80	35	115 (14.4)	17	6	23 (65.7)	97	41	138 (16.6)
High-risk	44	33	77 (9.6)	12	5	17 (48.6)	56	38	94 (11.3)
Low-risk	41	30	71 (8.9)	8	3	11 (31.4)	49	33	82 (9.8)
Uncharacterised	3	–	3 (0.4)	1	–	1 (2.9)	4	–	4 (0.5)
High-risk									
16	13	9	22 (2.8)	5	4	9 (25.7)	18	13	31 (3.7)
18	2	3	5 (0.6)	0	1	1 (2.9)	2	4	6 (0.7)
31	4	6	10 (1.3)	2	0	2 (5.7)	6	6	12 (1.4)
33	0	7	7 (0.9)	1	1	2 (5.7)	1	8	9 (1.1)
35	1	1	2 (0.3)	0	1	1 (2.9)	1	2	3 (0.4)
39	1	0	1 (0.1)	0	2	2 (5.7)	1	2	3 (0.4)
45	3	9	12 (1.5)	0	1	1 (2.9)	3	10	13 (1.6)
51	3	4	7 (0.9)	2	0	2 (5.7)	5	4	9 (1.1)
52	5	5	10 (1.3)	1	1	2 (5.7)	6	6	12 (1.4)
56	5	9	14 (1.8)	0	0	0 (0.0)	5	9	14 (1.7)
58	1	4	5 (0.6)	0	2	2 (5.7)	1	6	7 (0.8)
59	1	2	3 (0.4)	0	0	0 (0.0)	1	2	3 (0.4)
68	3	0	3 (0.4)	0	0	0 (0.0)	3	0	3 (0.4)
73	1	5	6 (0.8)	0	0	0 (0.0)	1	5	6 (0.7)
82	1	0	1 (0.1)	1	1	2 (5.7)	2	1	3 (0.4)
Low-risk									
6	2	1	3 (0.4)	0	0	0 (0.0)	2	1	3 (0.4)
11	1	1	2 (0.3)	0	1	1 (2.9)	1	2	3 (0.4)
26	0	2	2 (0.3)	0	0	0 (0.0)	0	2	2 (0.2)
30	0	0	0 (0.0)	3	0	3 (8.6)	3	0	3 (0.4)
40	0	2	2 (0.3)	0	1	1 (2.9)	0	3	3 (0.4)
42	11	7	18 (2.3)	0	0	0 (0.0)	11	7	18 (2.2)
43	0	2	2 (0.3)	0	1	1 (2.9)	0	3	3 (0.4)
44	1	0	1 (0.1)	0	0	0 (0.0)	1	0	1 (0.1)
53	0	4	4 (0.5)	0	0	0 (0.0)	0	4	4 (0.5)
54	0	1	1 (0.1)	0	0	0 (0.0)	0	1	1 (0.1)
55	1	1	2 (0.3)	0	0	0 (0.0)	1	1	2 (0.2)
61	1	0	1 (0.1)	0	0	0 (0.0)	1	0	1 (0.1)
66	7	1	8 (1.0)	0	0	0 (0.0)	7	1	8 (1.0)
67	1	1	2 (0.3)	0	0	0 (0.0)	1	1	2 (0.2)
70	3	2	5 (0.6)	0	0	0 (0.0)	3	2	5 (0.6)
81	0	1	1 (0.2)	0	0	0 (0.0)	0	1	1 (0.1)
83	1	6	7 (0.9)	1	1	2 (5.7)	2	7	9 (1.1)
84	1	0	1 (0.1)	0	0	0 (0.0)	1	0	1 (0.1)
86	1	0	1 (0.1)	0	0	0 (0.0)	1	0	1 (0.1)
Cp6108	0	1	1 (0.1)	0	0	0 (0.0)	0	1	1 (0.1)
Jc9710	2	4	6 (0.8)	0	0	0 (0.0)	2	4	6 (0.7)

four adenocarcinomas. The average age of cases was 56 years, ranging from 35 to 85 years. All but one case (99%) was high-risk HPV-positive. HPV16 was found in 65 (73.9%) cases, with the next most common types being HPV18 and 45 (5 cases each), and HPV31, 52 and 56 (3 cases each). Only one case was positive for multiple HPV types (HPV18 and 56). No low-risk types were detected in cervical cancer.

Upon comparison with HPV-positive women with normal cytology, women with cervical cancer showed a PR of 3.9 (95% CI: 2.4–6.2) to be infected with HPV16, and of 1.3 (95% CI: 0.8–2.5) to be infected with HPV18 (Table 3). All other high-risk types were much less common in cervical cancer than in HPV-positive women with normal cytology (PR = 0.4; 95% CI: 0.2–0.6 for any high-risk type other than HPV 16/18).

4. Discussion

A major finding of the present study, the first carried out in Poland, is the disclosure of a relatively important burden of HPV prevalence in the general female population, particularly in women below the age of 35 years. The age-standardised HPV prevalence (17.8%) in Warsaw was higher than in previous studies using the same HPV testing protocol in Italy and the Netherlands, but similar to that found in some parts of

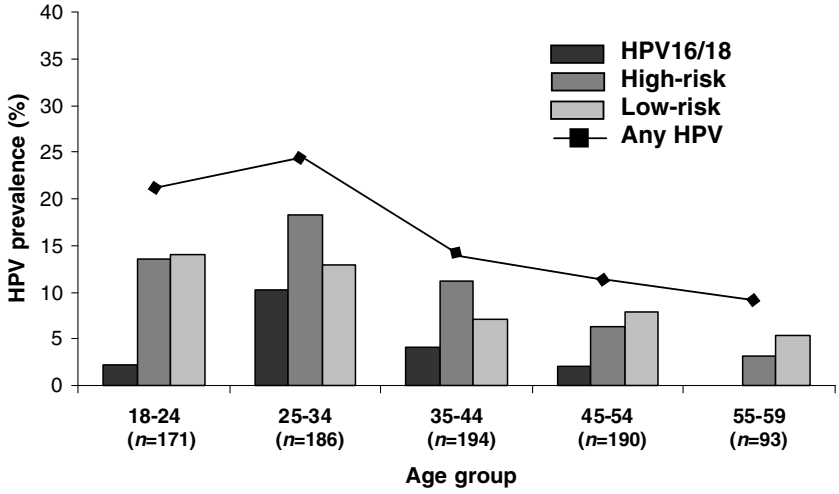

Fig. 1 – Age-specific prevalence of cervical human papillomavirus (HPV) DNA in the general female population (Warsaw, Poland, 2006).

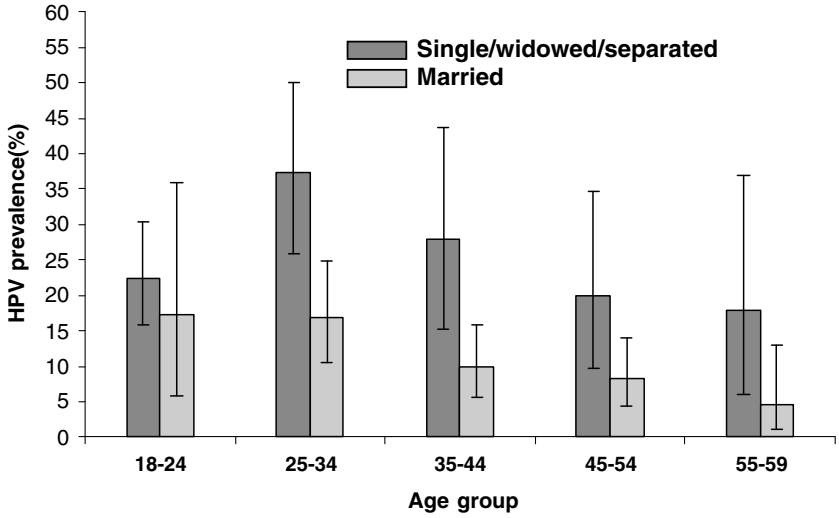

Fig. 2 – Age-specific prevalence of cervical human papillomavirus (HPV) DNA in the general population, by marital status (Warsaw, Poland, 2006).

South America and India.[6] In addition, the prevalence of high-risk HPV types in Poland can be compared with hybrid-capture 2 findings for primary cervical screening in different countries,[4] and were higher than among screened women in the United Kingdom, Germany and the Netherlands, but similar to those in France and the United States.[4] The elevated prevalence of high-risk HPV types can explain, along with the lack of organised screening programmes, the relatively high cervical cancer burden in Poland.[1]

HPV prevalence was similar in women aged 18–24 (21.6%) and 25–34 years (24.2%), declining thereafter. This age-specific profile is similar to the one reported in Turin, Italy,[15] but differs somewhat from those found in other high-resource countries in Europe,[7] Asia[7] and the USA[16] where a decrease in HPV positivity is already clear after age 25. In Poland, however, it was possible to show that HPV positivity doubled in unmarried women between the ages of 18–24 and 25–34 years, which was not seen for married women. A relatively late age at sexual debut (mean: 19.6 years), along with a tendency to delay marriage, may explain the persistence of relatively high HPV prevalence in women aged 25–34 years in some European countries like Poland.

As expected, lifetime number of sexual partners was the most important risk factor for HPV positivity in Warsaw,[17] reaching a relative risk of 8 for women reporting six or more lifetime sexual partners compared to women reporting only one. This association is the strongest that has been found in relation to HPV infection and number of sexual partners,[17] pointing to an accurate reporting of sexual activity in our study. The sexual behaviour of male partners was also confirmed to be an important risk factor for HPV prevalence. In agreement with previous results,[18] reproductive characteristics, oral contraceptives and condom use were not significantly associated with HPV positivity.

HPV16 was the most frequently detected type in the general female population in Warsaw, followed by HPV56, 45,

Table 2 – Detection of cervical human papillomavirus (HPV) DNA among 834 women from the general population, according to various characteristics (Warsaw, Poland, 2006)

Risk factors	N of women	HPV DNA pos. (%)	OR[a]	95% CI	OR[b]	95% CI
Age group (yrs)						
18–24	171	37 (21.6)	1	–	1	–
25–34	186	45 (24.2)	1.2	0.7–1.9	0.8	0.5–1.3
35–44	194	27 (13.9)	0.6	0.3–1.0	0.4	0.2–0.7
45–54	190	21 (11.1)	0.5	0.3–0.8	0.3	0.2–0.6
55–59	93	8 (8.6)	0.3	0.2–0.8	0.2	0.1–0.6
χ^2_1 *for trend*				$p = 0.002$		$p < 0.001$
Marital Status						
Married	509	55 (10.8)	1	–	1	–
Single	248	65 (26.2)	2.7	1.7–4.4	2.1	1.3–3.4
Widowed/separated	77	18 (23.4)	3.1	1.7–3.5	2.3	1.2–4.3
Age at sexual debut (yrs)						
⩾20	350	44 (12.6)	1	–	1	–
17–19	396	69 (17.4)	1.3	0.9–2.0	0.9	0.6–1.4
<17	79	24 (30.4)	2.3	1.3–4.2	1.2	0.6–2.4
χ^2_1 *for trend*				$p = 0.01$		$p = 0.82$
Lifetime number of sexual partners						
1	299	22 (7.4)	1	–		
2–3	279	42 (15.1)	2.4	1.4–4.3		
4–5	147	38 (25.9)	4.7	2.6–8.4		
⩾6	102	35 (34.3)	8.3	4.5–15.6		
χ^2_1 *for trend*				$p < 0.001$		
Husband's extramarital sexual relationships						
No	167	9 (5.4)	1	–	1	–
Uncertain	151	23 (15.2)	4.1	1.8–9.3	3.4	1.4–7.9
Yes	512	106 (20.7)	5.0	2.5–10.2	3.3	1.6–6.8
Parity						
Nulliparous	325	74 (22.8)	1	–	1	–
1	228	33 (14.5)	0.6	0.4–1.1	0.7	0.4–1.2
⩾2	280	30 (10.7)	0.5	0.3–0.9	0.8	0.4–1.3
χ^2_1 *for trend*				$p = 0.02$		$p = 0.32$

CI = confidence interval; OR = odds ratio.
a Adjusted for age.
b Adjusted for age and lifetime number of sexual partners, as appropriate.

Table 3 – Prevalence of selected human papillomavirus (HPV) types in 88 HPV-positive invasive cervical carcinomas (ICC) and 115 HPV-positive women with normal cytology (Warsaw, Poland, 2006–2007)

HPV type	ICC[ab] $n = 88$	Normal cytology HPV-positive $n = 115$	ICC: Normal cytology	
	n (%)	*n* (%)	Prevalence ratio	95% CI
16	65 (73.9)	22 (19.1)	3.9	2.4–6.2
18	5 (5.7)	5 (4.3)	1.3	0.8–2.5
Other high-risk types				
31	3 (3.4)	10 (8.7)	0.4	0.1–1.4
45	5 (5.7)	12 (10.4)	0.5	0.2–1.4
52	3 (3.4)	10 (8.7)	0.4	0.1–1.4
56	3 (3.4)	14 (12.2)	0.3	0.1–1.1
Any high-risk type other than 16/18[d]	17 (19.3)	51 (44.3)	0.4	0.2–0.6
Any low-risk type	0 (0)	71 (61.7)	0.0	0.0–0.1
⩾2 types	1 (1.2)[c]	35 (30.4)	0.04	0.0–0.4

CI = confidence interval.
a One HPV-negative ICC case included.
b Includes four adenocarcinoma, of which two were positive for HPV16 and two were positive for HPV18.
c HPV18 and 56.
d Includes HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82.

31, and 52. However, we confirmed that the type-distribution among this population was not representative of that in women with cervical cancer from the same region, with the relative importance of HPV16 being much greater in cervical cancer cases. HPV16 accounted for three-quarters of cervical cancers diagnosed in Warsaw. HPV18 accounted for similar proportions of HPV infection in women with and without cancer. Thus, HPV16 and 18 together accounted for 23% of HPV infections in women with normal cytology, but 79% of cervical cancer cases. This figure is somewhat higher than that reported among two smaller case-series of cervical cancer from Gdansk, Poland.[19,20] These findings highlight the varying potential for high-risk HPV types to cause cancer and thus the importance to consider type distribution among invasive cancers when estimating the theoretical fraction of cervical cancer preventable by current or future HPV vaccines.[21] Furthermore, although multiple infections were frequent in the general population, 98% of cervical cancers were infected with a single HPV type, highlighting the selection of one high-risk type in the course of malignant transformation.

Strengths of the present study include a relatively large sample size (especially among young and unmarried women) and the use of a standardised and well-validated HPV test allowing comparisons with similar studies around the world.[6] The main limitation was the substantial number of women from the general population who refused to participate. However, as HPV infection is asymptomatic and was not associated with socio-economic class in the present, or many previous studies,[22,23] it is unlikely that HPV positivity and lack of participation were strongly correlated. Finally, our present findings may not be completely representative of the Polish female population living outside the country's capital, although current data suggest that cervical cancer incidence rates are uniformly high across the whole of Poland.[1]

Conflict of interest statement

None declared.

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REFERENCES

1. Parkin DM, Whelan SL, Ferlay J, Thomas DB, Teppo L, editors. Cancer incidence in five continents, Vol. VIII. IARC Scientific Publications No. 155. Lyon: International Agency for Research on Cancer; 2002.
2. Levi F, Lucchini F, Negri E, Franceschi S, La Vecchia C. Cervical cancer mortality in young women in Europe: patterns and trends. *Eur J Cancer* 2000;**36**:2266–71.
3. Rekosz M, Karska A, Osiecka-Tesny E, Jackowska A, Wojcinska M. 15 years of active cervical cancer screening done by the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw - results obtained to-date. *Nowotwory Journal of Oncology* 2003;**53**:62–9.
4. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006;**119**:1095–101.
5. Kahn JA, Burk RD. Papillomavirus vaccines in perspective. *Lancet* 2007;**369**:2135–7.
6. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet* 2005;**366**:991–8.
7. Franceschi S, Herrero R, Clifford GM, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. *Int J Cancer* 2006;**119**:2677–84.
8. Kornya L, Cseh I, Deak J, Bak M, Fulop V. The diagnostics and prevalence of genital human papillomavirus (HPV) infection in Hungary. *Eur J Obstet Gynecol Reprod Biol* 2002;**100**:231–6.
9. Syrjanen S, Shabalova I, Petrovichev N, et al. Sexual habits and human papillomavirus infection among females in three New Independent States of the former Soviet Union. *Sex Transm Dis* 2003;**30**:680–4.
10. Snijders PJF, van den Brule AJC, Jacobs MV, Pol RP, Meijer CJLM. HPV DNA detection and typing in cervical scrapes by general primer GP5+/6+ PCR. In: *Methods in molecular medicine Volume 119: Human papillomaviruses - methods and protocols*. Totowa: Humana Press; 2005. p. 101–14.
11. Roda Husman AM, Snijders PJ, Stel HV, van den Brule AJ, Meijer CJ, Walboomers JM. Processing of long-stored archival cervical smears for human papillomavirus detection by the polymerase chain reaction. *Br J Cancer* 1995;**72**:412–7.
12. Jacobs MV, Walboomers JM, Snijders PJ, et al. Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer* 2000;**87**:221–7.
13. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;**40**:779–87.
14. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;**348**:518–27.
15. Ronco G, Ghisetti V, Segnan N, et al. Prevalence of human papillomavirus infection in women in Turin, Italy. *Eur J Cancer* 2005;**41**:297–305.
16. Giuliano AR, Papenfuss M, Abrahamsen M, et al. Human papillomavirus infection at the United States-Mexico border: implications for cervical cancer prevention and control. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:1129–36.
17. Vaccarella S, Franceschi S, Herrero R, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:326–33.
18. Vaccarella S, Herrero R, Dai M, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:2148–53.
19. Liss J, Lukaszuk K, Gulczynski J, et al. The incidence of human papilloma virus (HPV) DNA in patients with cervical carcinoma from Gdansk region. *Ginekol Pol* 2002;**73**:740–4.

20. Dybikowska A, Licznerski P, Podhajska A. HPV detection in cervical cancer patients in northern Poland. *Oncol Rep* 2002;**9**:871–4.
21. Franceschi S, Clifford GM. Re: A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J Natl Cancer Inst* 2005;**97**:938–9.
22. Shin HR, Lee DH, Herrero R, et al. Prevalence of human papillomavirus infection in women in Busan, South Korea. *Int J Cancer* 2003;**103**:413–21.
23. Sukvirach S, Smith JS, Tunsakul S, et al. Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. *J Infect Dis* 2003;**187**:1246–56.