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Retrospective Study of the Prevalence of High-Risk Human Papillomaviruses among Croatian Women

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A B S T R A C T

The infection with Human papillomavirus (HPV) is the necessary cause for cervical cancer. There are at least 15 High-Risk (HR) HPV types that are significantly associated with progression of cervical intraepithelial neoplasia to cervical cancer. Since previous studies showed that the prevalence of HPV in cervical cancers varies among different geographic regions, we wanted to investigate the prevalence of HPV types in Croatia, especially low abundant HR HPV types. By means of consensus primers directed polymerase chain reaction (PCR), we analysed cervical DNA samples of 2,136 Croatian women, mostly with abnormal cervical smears, in order to detect the presence of HPV. Type-specific primers were then used to determine Low-Risk (LR) HPV types 6/11 and HR HPV types 16, 18, 31, 33, 45, 52 and 58. Out of 2,136 specimens, 1,255 (58.8%) were positive for HPV. More than half of positive samples were typed (64.5%) and 35.5% still remained untyped. Multiple HPV infections were found in 10.3% of the cases. The most prevalent type, including both single and multiple infections, was HPV 16 with the prevalence of 15.9%, followed by HPV types 31, 6/11, 33, 18, 52, 45 and 58 with 8.7%, 7.1%, 4.5%, 3.8%, 2.3%, 1.2% and 1.1%, respectively. The significant increase of frequency from Low-grade Squamous Intraepithelial Lesions (LSIL) to High-grade Squamous Intraepithelial Lesions (HSIL) was observed for HR HPV types 16, 18, 31 and 33 but not 45, 52 and 58. The frequency of unknown HPV types was almost the same in cervical specimens of women with LSIL and those with HSIL, 19.8% and 21.1%, respectively. The prevalence of HPV infection rate decreased significantly with patient age from 68.5% (age group 12 to 24 years) to 38.8% (age group 45 to 54 years). But, in women aged 55 or older the overall prevalence increased to 56.6%. Our results indicate that prevalence of HR HPV types in Croatia is similar to other countries. We suggest that HPV positive women in Croatia should be closely monitored by typing for HR HPV types: 16, 18, 31, 33, 45, 52 and 58.

Key words: *human papillomavirus (HPV), high-risk (HR) types, Croatia*

Introduction

Cervical cancer is the second most common malignancy after breast cancer among women worldwide. More than 80% of cases occur in developing countries. In 2002, about 493,000 cervical cancer cases (incidence rate 16/100,000) and about 273,000 deaths from cervical cancer (mortality rate 8.9/100,000), respectively, were recorded worldwide[1]. The incidence and mortality rates in Croatia in 2002 represents the intermediate value of the highest and lowest rates noted in Europe, with 431 cases (incidence rate 18/100,000) and 209 deaths (mortality rate 8.7/100,000) from cervical cancer, respectively[2,3]. This indicates that cervical cancer represents a major health concern in Croatia that needs to be improved.

Epidemiological studies have clearly established human papillomavirus (HPV) infection as the prerequisite for cervical cancer[4]. Studies in 22 countries coordinated by the International Agency for Research on Cancer (IARC), identified HPV DNA in almost all (99.7%) of about 1,000 cases of cervical cancer[5]. To date, more than 200 different HPV types have been recognized on the basis of DNA sequence data[6]. More than 100 HPV genotypes are well characterized[7]. Over 40 types infect the human anogenital area, of which some can cause genital warts and others can lead to precursor cervical lesions, CIN (cervical intraepithelial neoplasia) and cervical cancer[7]. At least 15 HPV types are significantly associ-

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ated with progression of CIN to cervical cancer and are considered carcinogenic and named High-Risk (HR) HPV types[8]. Thus, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 were classified as HR or carcinogenic types, HPV 26, 53 and 66 as probable carcinogenic types, while HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108 as Low-Risk (LR)[8]. In contrast to HR HPV types, LR types are associated with benign genital warts and only occasionally found in cervical carcinomas. According to the epidemiological and the phylogenetic studies, most HR HPV types associated with lesions of similar pathologies are phylogenetically related to either HPV 16 (31, 33, 35, 52 and 58) or HPV 18 (39, 45, 59 and 68)[7].

The prevalence of HPV types in cervical cancers varies among different geographic areas[4,9–10]. In a worldwide survey, HPV 16 was found to be the most prevalent type in cervical cancer (51.0%), followed by HPV 18 (16.2%) and other types such as HPV 45, 31, 33, 58 and 52 (collectively accounting for 18.3% cases)[4]. In Europe, beside HPV 16, types 18, 33, 31 and 45 were the most frequently found HR HPV types in cervical cancer[4]. Infection with HPV 45 was found to be third most frequent in Africa, after HPV 16 and 18. HPV 52 and 58 are less frequent in Europe, but not uncommon worldwide, especially in Asia, being the most frequently found HR HPV types after 16 and 18[4].

There is limited data regarding the prevalence of low abundant HR HPV types in Croatia. Therefore, the aim of this study was to evaluate the prevalence of HR HPV types 45, 52 and 58 among Croatian women on the archival DNA samples where HPV types 6/11, 16, 18, 31 and 33 were previously determined[11,12].

Material and Methods

Study population

Two thousand one hundred and thirty six Croatian women, mostly with abnormal cervical smears, were enrolled in this study. The cervical specimens for HPV detection and typing were collected in different gynaecological hospital clinics in Zagreb, Croatia[11,12]. The cytological diagnosis[13] of the study population consisted of 0.7% (15/2,136) normal Pap smears, 24.8% (529/2,136) ASCUS (atypical squamous cells of undetermined significance), 28.8% (615/2,136) LSIL (Low-grade Squamous Intraepithelial Lesions) and 40.9% (874/2,136) HSIL (High-grade Squamous Intraepithelial Lesions). Furthermore, there were 4.8% (103/2,136) women with unknown cytological diagnosis.

DNA preparation

DNA from cervical cell samples was isolated as described previously[11,12]. Briefly, cervical cell suspensions were collected and treated with proteinase K (100 μg/ml in lysis buffer, 10 mM Tris-HCl; pH 7.5, 1 mM EDTA, pH 7.9; 0.5% SDS) overnight at 37°C or 2h at 56°C. Standard phenol-chloroform extraction and ethanol precipitation were used for DNA purification. Pelleted DNA was resuspended in 50–100 μL of deionised sterile water and stored at –20°C until further analysis[14]. In order to determine the quality and the quantity of isolated DNA, each DNA was analysed by electrophoresis on 1% agarose gels stained with ethidium bromide and spectrophotometrically[14].

HPV detection and typing

Cervical DNA samples were tested for the presence of HPV DNA by PCR based method as previously described[11,12–15]. Briefly, two sets of consensus primers: MY09/MY11, degenerated primers, and L1C1/L1C2-1/L1C2-2 primers were used for HPV DNA detection. To internally control the quality of the target DNA and the absence of PCR inhibitors, β-globin specific primers were used in the multiplex PCR with MY09/MY11 primers. Type-specific primers for HPV 6/11, 16, 18, 31 and 33 were also used in two separate multiplex PCRs, i.e. HPV 6/11 with 31 and HPV 16, 18 with 33. All amplification reactions were performed on 100 ng of each DNA in a total volume of 20 μl under the optimized conditions as previously described[11,12–15].

In this study, archival DNA samples were additionally examined for the presence of HPV 45, 52 and 58 with type-specific primers directed PCR, also [5,16]. The reaction mixtures contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM of each dNTP, 0.15 μM of each type-specific primer, 0,12 U AmpliTaq Gold DNA Polymerase (Roche) and 100 nm of each DNA in a total volume of 20 μl. The amplification conditions for HPV 52 and 58 consisted of 30 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 30 sec, while for HPV 45 it consisted of 35 cycles of denaturation at 95°C for 40 sec, annealing at 55°C for 40 sec and extension at 72°C for 45 sec, with in both cases an initial denaturation at 95°C for 10 min and a final extension of 7 min.

Aliquots (10 μl) of each PCR product were resolved by electrophoresis in a 2% agarose gel stained with ethidium bromide[14]. The amplified products were identified by UV irradiation of the gels, and photographed by Image Master VDS (Pharmacia Biotech).

Statistical analysis

The standard Chi-square (χ^2) test was used. Two-tailed p values were calculated in 2 x 2 tables using the GraphPad Prism (version 4.00) (GraphPad Software, San Diego, California). All tests were two sided and the significance level was set at $p < 0.05$[17].

Results

The average age of the study population was 30.6 (ranging from 12 to 75 years of age). The distribution of patient cytological diagnosis according to patient age is shown in Figure 1.

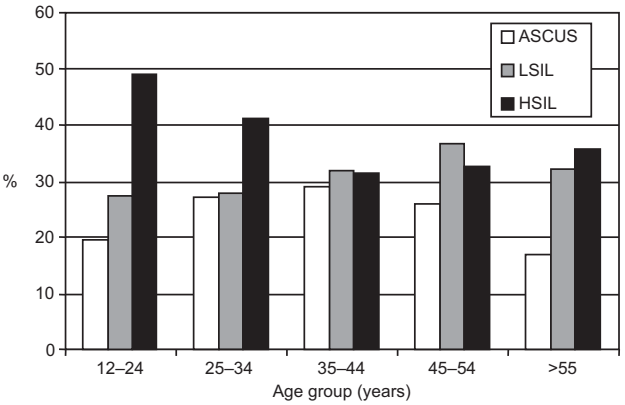

Fig. 1. Distribution of patient diagnosis according to patient age. ASCUS – atypical squamous cells of undetermined significance, LSIL – low-grade squamous cell intraepithelial lesion, HSIL – high-grade squamous cell intraepithelial lesion.

Distribution of different HPV types

All DNA specimens prepared from cervical cell samples were β-globin positive and as such suitable for further HPV DNA detection and typing by PCR. Out of 2,136 DNA samples, 1,255 (58.8%) were positive for any HPV type (Tables 1 and 2).

More than half (64.5%) of HPV positive samples were typed. In 445 (35.5%) samples HPV type was not determined (HPV X). Multiple HPV infections with two or more HPV types were found in 10.3% (129/1,255) of the cases, mainly among younger women (mean age 26) (Table 2).

LR HPVs (types 6 or 11) appeared as single infections only in 4.9% (104/2,136) cases. Among HR HPVs, type 16 was the most frequently found as single infection in 11.9% (254/2,136) of the cases, followed by HPV types 31, 33, 18, 52, 45 and 58 found in 5.7% (121/2,136), 2.8% (60/2,136), 2.6% (55/2,136), 2.2% (46/2,136), 1.0% (22/2, 136), and 0.9% (19/2,136) of the cases, respectively (Tables 1 and 2).

Out of 129 multiple HPV infections (Table 3), there were 82 (63.6%) co-infections of two or three HR HPVs and 47 (36.4%) co-infections of LR HPV (type 6 or 11) and one or two HR HPV types. The co-infection of HPV types 16 and 31 or 16 and 6/11 were the most frequent, present in 25.6% (33/129) and 19.4% (25/129) cases, respectively.

Prevalence of different HPV types

HPV 16 was present in 85 multiple infections and in 254 single infections, which increases the total prevalence of HPV 16 in the study population to 15.9% (339/2, 136). HPV 31 was the second most frequent HPV type, being represented in 65 multiple infections and 121 single infections, with a final prevalence of 8.7% (186/2,136) (Figure 2). The prevalence, including both single and multiple infections, of HPV types 6/11, 18, 33, 45, 52 and 58 was 7.1% (151/2,136; not shown), 3.8% (82/2,136), 4.5% (95/2,136), 1.2% (25/2,136), 2.3% (49/2,136) and 1.1% (23/2,136), respectively (Figure 2).

TABLE 1
HPV TYPE SPECIFIC PREVALENCE IN THE GROUP OF PATIENTS WITH DIFFERENT CYTOLOGICAL FINDINGS

HPV type	Cytological diagnosis										Total	
	Unknown		Normal		ASCUS		LSIL		HSIL			
	N	%	N	%	N	%	N	%	N	%	N	%
HPV 6/11	5	4.9	0	0.0	29	5.5	29	4.7	41	4.7	104	4.9
HPV 16	10	9.7	0	0.0	49	9.3	46	7.5	149	17.1	254	11.9
HPV 18	3	2.9	0	0.0	8	1.5	13	2.1	31	3.6	55	2.6
HPV 31	5	4.9	0	0.0	26	4.9	21	3.4	69	7.9	121	5.7
HPV 33	3	2.9	0	0.0	7	1.3	15	2.4	35	4.0	60	2.8
HPV 45	0	0.0	0	0.0	8	1.5	6	1.0	8	0.9	22	1.0
HPV 52	1	1.0	0	0.0	15	2.8	11	1.8	19	2.2	46	2.2
HPV 58	0	0.0	0	0.0	3	0.6	7	1.1	9	1.0	19	0.9
Any HR	22	21.4	0	0.0	116	21.9	119	19.4	320	36.6	577	27.0
HPV X	17	16.5	3	20.0	119	22.5	122	19.8	184	21.1	445	20.8
Multiple	7	6.8	1	6.7	22	4.2	28	4.6	71	8.1	129	6.0
Positive	51	49.5	4	26.7	286	54.1	298	48.5	616	70.5	1,255	58.8
Negative	52	50.5	11	73.3	243	45.9	317	51.5	258	29.5	881	41.3
Total	103	4.8	15	0.7	529	24.8	615	28.8	874	40.9	2,136	100

ASCUS – atypical squamous cells of undetermined significance, LSIL – low grade squamous cell intraepithelial lesion, HSIL – high grade squamous cell intraepithelial lesion, HPV – human papillomavirus, HR – high-risk, HPV X –unknown HPV type

TABLE 2
HPV TYPE SPECIFIC PREVALENCE BY AGE

HPV type	Age group (years)												Total	
	Unknown		12–24		25–34		35–44		45–54		≥55			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HPV 6/11	5	8.6	44	6.4	33	4.2	9	2.4	10	5.6	3	5.7	104	4.8
HPV 16	11	19.0	97	14.0	96	12.3	35	9.4	9	5.1	6	11.3	254	11.9
HPV 18	2	3.5	16	2.3	27	3.5	5	1.3	3	1.7	2	3.8	55	2.6
HPV 31	3	5.2	55	8.0	42	5.4	14	3.7	4	2.3	3	5.7	121	5.7
HPV 33	1	1.7	25	3.6	20	2.6	9	2.4	3	1.7	2	3.8	60	2.8
HPV 45	0	0.0	3	0.4	13	1.7	5	1.3	1	0.6	0	0.0	22	1.0
HPV 52	1	1.7	11	1.6	23	2.9	6	1.6	5	2.8	0	0.0	46	2.2
HPV 58	0	0.0	7	1.0	9	1.2	2	0.5	0	0.0	1	1.9	19	0.9
Any HR	18	31.0	214	30.9	230	29.5	76	20.3	25	14.0	14	26.4	577	27.0
HPV X	13	22.4	153	22.1	164	21.0	72	19.3	31	17.4	12	22.6	445	20.8
Multiple	3	5.2	63	9.1	53	6.8	6	1.6	3	1.7	1	1.9	129	6.0
Positive	39	67.2	474	68.5	480	61.5	163	43.6	69	38.8	30	56.6	1,255	58.8
Negative	19	32.8	218	31.5	301	38.5	211	56.4	109	61.2	23	43.4	881	41.3
Total	58	2.7	692	32.4	781	36.6	374	17.5	178	8.3	53	2.5	2,136	100

HPV – human papillomavirus, HR – high-risk, HPV X – presence of unknown HPV type

TABLE 3
MULTIPLE HPV INFECTIONS

HPV types	Cases (N)	%
16, 31	33	25.6
6/11, 16	25	19.4
16, 33	14	10.9
18, 31	11	8.5
6/11, 31	7	5.4
31, 33	6	4.7
6/11, 18	5	3.9
6/11, 33	5	3.9
16,18	4	3.1
16, 31, 33	3	2.3
18, 33	2	1.6
45, 52	2	1.6
6/11, 31, 33	2	1.6
16, 18, 31	2	1.6
16, 58	1	0.8
33, 58	1	0.8
45, 58	1	0.8
52, 58	1	0.8
6/11, 16, 18	1	0.8
6/11,16, 31	1	0.8
6/11, 18, 33	1	0.8
16, 18, 33	1	0.8
Total	129	100

HPV – human papillomavirus

Prevalence of HR HPV types in HSIL

When both single and multiple infections are included HPV 16 was present in 23% (201/874) of HSIL samples HPV types 31, 33, 18, 52, 58 and 45 were found in 12.0% (105/874), 6.1% (53/874), 5.0% (44/874), 2.3% (20/874), 1.1% (10/874) and 0.9% (8/874) of the cases, respectively (Figure 3).

HPV distribution according to patient diagnosis

The prevalence of HPV increased significantly (χ^2 = 74, d.f. = 1, $p < 0.0001$) from 48.5% to 70.5% with the severity of cytological findings (Table 1 and Figure 4). While LR HPV types 6/11 were equally distributed among LSIL (4.7%) and HSIL (4.7%), HR HPVs were almost two times more frequent in HSIL (36.6%) than in LSIL (19.4%) (for presence of any HR HPV, χ^2 = 51.75, d.f. = 1, $p < 0.0001$). HPV type 16, as a single infection, was the most frequent, found in 7.5% of cases in LSIL compared to 17.1% in HSIL (χ^2 = 29.0, d.f. = 1, $p < 0.001$). Multiple HPV infections were also two times more frequent in HSIL than in LSIL, 8.1% and 4.6%, respectively. The frequency of unknown HPV types (HPV X) was almost the same in cervical specimens of women with LSIL and those with HSIL, 19.8% and 21.1%, respectively.

HPV distribution according to patient age

The frequency of HPV infections decreased significantly (χ^2 = 94.2, d.f. = 4, $p < 0.0001$) with patient age from 68.5% (age group 12 to 24 years) to 38.8% (age group 45 to 54 years) (Table 2 and Figure 5). But, the prevalence of HPV in women aged 55 or older increased

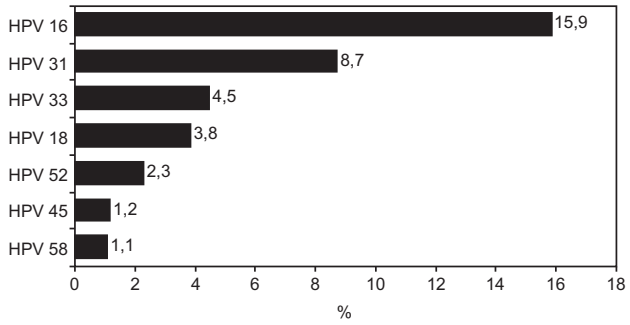

Fig. 2. Prevalence of specific high-risk human hapillomavirus (HPV) types including single and multiple infections in the whole study population.

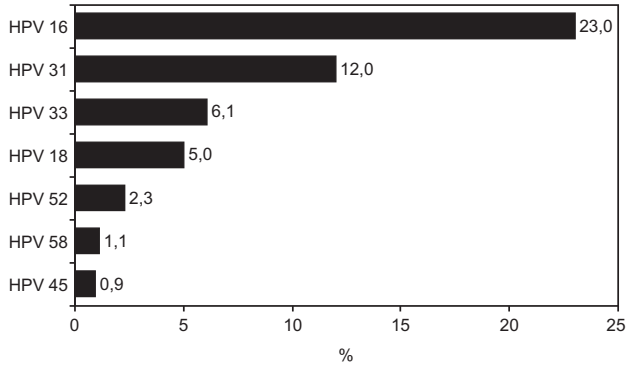

Fig. 3. Prevalence of specific high-risk human papillomavirus (HPV) types including single and multiple infections in high--grade squamous cell intraepithelial lesion (HSIL) samples.

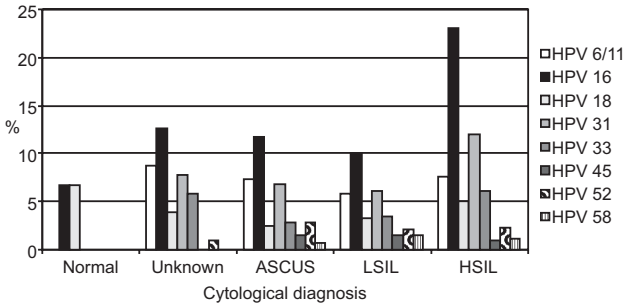

Fig. 4. Prevalence of human hapillomavirus (HPV) types including single and multiple infections according to cytological diagnosis. Unknown – diagnosis not known, ASCUS – atypical squamous cells of undetermined significance, LSIL – low-grade squamous cell intraepithelial lesion, HSIL – high-grade squamous cell

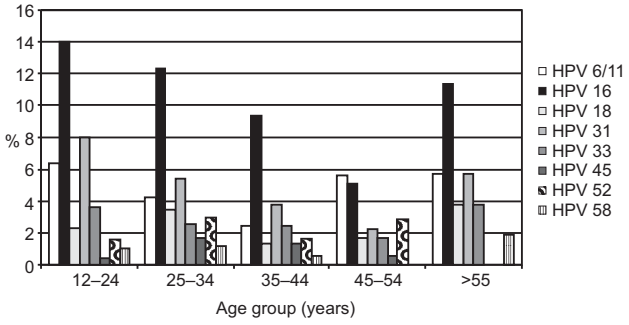

Fig. 5. Age dependent prevalence of human hapillomavirus (HPV) types including single and multiple infections.

to 56.6%. The observed trend is statistically significant for HPV 16 ($\chi^2 = 10$, d.f. = 1, p = 0.0013), HPV 31 ($\chi^2 = 10$, d.f. = 1, p = 0.0013), presence of any HR HPV ($\chi^2 = 22$, d.f. = 1, $p < 0.0001$) and presence of multiple infection ($\chi^2 = 28$, d.f. = 1, $p < 0.0001$).

Discussion

This study was designed to investigate the prevalence of low abundant HR HPV types 45, 52 and 58 among Croatian women. We analysed archival DNA samples from 2,136 cervical specimens collected from Croatian women that were previously tested for the presence of LR HPV types 6 or 11 and HR HPV types 16, 18, 31 and 33[11,12,15].

The overall detection of HPV was 58.8%. Out of 1,255 positive samples, 64.5% were typed and in 35.5% of samples the type was not determined. Those samples gave positive results with general primers, but they were negative with type-specific primers used in this study.

The geographic variation of the distribution of different HPV types is well established in some regions[4,18–20]. Worldwide, in squamous cell carcinoma HPV 16 was the most prevalent type varying from 46% in Asia to 63% in Europe, while HPV 18 was the second most common HPV type. HPV 33, 31 and 45 in Europe and HPV 45 in Africa were the third HPV types most frequently associated to cervical squamous cell carcinoma. HR HPV 58 and 52 were found to be less frequent in Europe, but not uncommon worldwide, especially in Asia, where these two types took the third and fourth place after HPV 16 and 18.

Herein, we found that HPV 16 was the most prevalent type among Croatian women, as expected, and it was present in 15.9% cases of both single and multiple HPV infections. HPV 31 was the second most prevalent HPV type appearing in 8.7% both single and multiple HPV infections, followed by HR HPVs 33, 18, 52, 45 and 58 (Figure 2). The distribution of HR HPV types in the precancerous lesions is slightly different than those in cervical cancer cases. According to a meta-analysis of Clifford et al.[21] overall HPV prevalence was higher in squamous cell carcinoma of the cervix (SCC) than in HSIL, 87.6% *versus* 84.2%, respectively (SCC : HSIL ratio 1.04). HPV 16 was found to be the most common type in both SCC (54.3%) and HSIL (45.0%), with a ratio of 1.21. HPV 18 was also more prevalent in SCC (12.6%) than in HSIL (7.0%), with a ratio of 1.79. Interestingly, HPV 45 was the third type with a higher ratio (1.85) than those observed with HPV 16 and 18. It was also more frequent in SCC (4.2%) than in HSIL (2.3%). All other analysed HR HPVs (including HPV 31, 33, 52 and 58) had ratios less than 1, i.e. between 0.1 and 0.6[21]. These results suggested

that HSIL, when associated with HPV 16, 18 or 45 is more likely to progress to SCC than HSIL associated with other HR HPV types[10,21]. This evidence pointed out that women infected with HPV 16, 18 or 45 may require closer surveillance than women infected with other HR HPV types.

When we compared our prevalence analysis of HR HPV types in HSIL (including both single and multiple infections) with a worldwide meta-analysis of Clifford et al.[21], we found some discrepancies (Figure 3). HPV 16 was, as expected, the most frequently found type in HSIL samples in both studies but with almost twice lower prevalence in our study than those of Clifford et al., 23% and 45%, respectively. Similarly in both studies, HPV 31 was on the second place with the prevalence of 12.0% in our study *versus* 8.8% in Clifford et al. study. HPV 33, followed these two HPV types in both studies with, in this case, similar prevalence of 6.1% in this study and 7.2% in Clifford et al. study. Further, we found slightly lower incidence of HPV 18 in HSIL samples, in 5.0% cases *versus* 7.1%. The major discrepancy was the prevalence of HPV 58. While it was rather frequently found type (6.9%) in Clifford et al. study, we found much lower incidence among Croatian women (1.1%). We also noticed unexpected low prevalence for HPV 52 (2.3%) and HPV 45 (0.9%) compared to Clifford et al. study (5.2% and 2.3%, respectively). These discrepancies could be explained by (1) low number of analysed samples in this study, (2) different determination of cytological diagnosis HSIL[13], (3) the choice of primers sets for type-specific HPV amplification[5,12,16] or (4) our data represent a real picture of HPV prevalence among Croatian women. However, these observations clearly point out that further analysis with larger number of samples and alternative method of typing need to be carried out in the future to clarify this dilemma.

Herein, we found a strong correlation between the distribution of HR HPV infections and the severity of cervical lesions, i.e. the frequency of HR HPVs (type 16, 18, 31 and 33) increased with increasing severity of squamous intraepithelial lesions-SIL, from 19.4% in LSIL to 36.6% in HSIL ($\chi^2 = 74$, d.f. = 1, $p < 0.0001$). HPV 16 is the most predictive virus type of SIL and it is present in 7.5% cases of LSIL and 17.1% cases of HSIL ($\chi^2 = 29$, d.f. = 1, $p < 0.0001$). This result is not surprising, and matches data found in previous reports[22,23–24]. The progression from LSIL to HSIL is strongly associated with the presence of HR HPVs, while no progression is associated with the presence of LR HPV types or the absence of HPV[25], which further stresses the importance of HPV DNA typing.

The frequency of unknown HPV types (HPV X) was approximately the same in cervical swabs of women with LSIL and those with HSIL, 19.8% versus 21.1%, respectively. Although type-specific primers that we used in this study were detecting mainly group of HR HPV types expected in Europe, we would like to continue our study and determine the unknown HPV types[11,12].

In this study, all 129 multiple HPV infections contained at least one HR HPV type. There were 63.6% co-infections of two or three HR HPV types, while 36.4% co-infections of LR and HR HPV types (Table 3). The co-infection of HPV types 16 and 31 was most frequently found followed by the co-infection of HPV 16 and 6/11. The frequency of co-infections follows the frequency of individual HPV types found in the study population (Figures 2 and 3). HPV types 45, 52 and 58 were rarely found in multiple infections.

Herein, multiple infections were two times more frequent in HSIL than in LSIL, 8.1% *versus* 4.6%, what is not surprising because multiple HPV infections were mainly represented by co-infections of two or three HR HPVs or HR- and LR HPVs. In the similar study population in Taiwan, Chang et al.[26] found adverse results, i.e. decreasing frequency of multiple HPV infections with increasing severity of cervical lesions. However, a similar decrease of multiple infection frequency can be observed from CIN 2 to CIN 3, if the HSIL category is classified accordingly to the Croatian classification of cervical smears[13] (data not shown). In both cases, the HPV infections associated with LSIL still represents transient infections, while the HPV infections associated with HSIL, especially CIN 3 most probably persistent infections[27].

In this study, the frequency of HPV infections decreased significantly (χ^2 for trend = 72.42, d.f. = 1, $p < 0.0001$) with patient age. Women under the age of 24 years exhibited the highest rate of HPV infections (68.5%), while women between 45 and 54 years of age exhibited the lowest rate of HPV infections (38.8%). Multiple HPV infections were also found mainly among younger women (mean age 26). This trend of high prevalence in young age after the beginning of sexual activity and then a steady decline in subsequent age group is observed in other studies[27]. In this study, an increase of HPV prevalence in women aged 55 or older to 56.6% was also observed. This trend of gradual fall and then increase in older age has been observed for any HR HPV, particularly HPV 16, HPV 18, 31 and 33 but not 45, 52 and 58, and multiple HPV infections (Table 2 and Figure 5). Interestingly, HPV 45 and 52 were not found among women ≥ 55, while HPV 58 was found mostly in the age group ≥ 55 but absent in the age group 45–54 years of age; the reason of that observation could be a small number of analysed specimens in these age groups. Further analysis with larger number of samples is needed in these age groups.

In conclusion, the distribution of HPV infections among Croatian women is similar to most countries with HPV 16 being the most prevalent type followed by HPV 31 in precancerous lesions. HPV 33, 18 and 52 are all at the third place of prevalence in this study group, while HPV 45 and 58 remain low abundant HR HPV types. As HPV detection of HR types is recommended for women with equivocal cytology and for follow-up after treatment of high-grade cervical lesions according to the Croatian diagnostic and therapeutic guidelines for management of women with premalignant cervical lesions[28], we suggest to perform HPV typing for HR HPV types: 16, 18, 31, 33, 45, 52 and 58 in HPV positive women in order to improve the monitoring process and prevent the cancer development.

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RETROSPEKTIVNA ANALIZA UČESTALOSTI VISOKORIZIČNIH TIPOVA HUMANOG PAPILOMAVIRUSA (HPV) KOD ŽENA U HRVATSKOJ

SAŽETAK

Infekcija humanim papilomavirusom (HPV) glavni je uzrok raka vrata maternice. Postoji najmanje 15 visokorizičnih tipova HPV-a koji su značajno povezani s napredovanjem cervikalne intraepitelne neoplazije (CIN) prema raku vrata maternice. Budući da su prijašnje studije pokazale da je učestalost HPV-a različita među različitim zemljopisnim područjima, naš cilj je bio ispitati učestalost tipova HPV-a u Hrvatskoj, posebno rijetkih visokorizičnih tipova HPV-a. Metodom lančane reakcije polimerazom (PCR), pomoću konsenzus početnica, a kako bismo ustanovili prisutnost infekcije HPV-om, analizirali smo uzorke DNK 2.136 žena u Hrvatskoj, uglavnom s abnormalnim nalazom brisa vrata maternice. Tip-specifične početnice korištene su za detekciju sljedećih tipova HPV-a: niskorizičnih 6/11 te visokorizičnih 16, 18, 31, 33, 45, 52 i 58. Od ukupno 2.136 uzoraka, 1.255 (58,8%) je bilo pozitivno, a 881 (41,3%) uzorak je bio negativan na prisutnost nekog tipa HPV-a. Više od polovine pozitivnih uzoraka bilo je tipizirano (64,5%), dok kod 35,5% nije utvrđen tip HPV-a. Višestruke infekcije HPV-om su pronađene u 10,3% slučajeva. Utvrđeno je da je najučestaliji tip HPV 16 s učestalošću od 15,9%, uključujući jednostruke i višestruke infekcije. Slijedili su ga tipovi HPV-a: 31, 6/11, 33, 18, 52, 45 te 58 s 8,7%, 7,1%, 4,5%, 3,8%, 2,3%, 1,8% te 1,1% učestalosti. Uočeno je značajno povećanje učestalosti od blagih (LSIL, engl. Low-grade Squamous Intraepithelial Lesions) do težih (HSIL, High-grade Squamous Intraepithelial

Lesions) promjena vrata maternice za visokorizične tipove HPV-a 16, 18, 31 i 33, ali ne i za HPV 45, 52 i 58. Učestalost nepoznatih tipova HPV-a (HPV X) bila je gotovo jednaka u uzorcima vrata maternice žena sa citološkom dijagnozom LSIL i HSIL, 19,8% i 21,1%. Uočeno je značajno smanjenje broja HPV-a s porastom dobi ispitanica, od 68,5% (dobna skupina od 12 do 24 godine) prema 38,8% (dobna skupina od 45 to 54 godine). Međutim, kod žena od 55 godina i starijih ukupna učestalost infekcija HPV-om je porasla na 56,6%. Ovi rezultati učestalosti visokorizičnih tipova HPV-a u Hrvatskoj se podudaraju s onima zabilježenim u drugim zemljama. Smatramo važnim HPV-pozitivne žene u Hrvatskoj tipizirati barem na prisustvo visokorizičnih tipova HPV-a: 16, 18, 31, 33, 45, 52 i 58 kako bi im se pružila maksimalna pozornost.