Prevalence of Human Papillomavirus Types 16, 18, and 45 in Women With Cervical Intraepithelial Changes: Associations With Colposcopic and Histological Findings

Kristina Jarienė, Daiva Vaitkienė, Arnoldas Bartusevičius, Eglė Tvarijonavičienė, Meilė Minkauskienė, Rūta Nadišauskienė, Valdemaras Kruminis, Mindaugas Kliučinskas
Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, Lithuania

Key words: *high-risk human papillomavirus; prevalence; cervical intraepithelial changes; colposcopy; biopsy.*

Summary. *The aim of the study was to determine the prevalence of human papillomavirus (HPV) types 16, 18, and 45 in women with cervical intraepithelial changes caused by high-risk HPV in relation to colposcopic and histological findings.*

Material and Methods. A prospective study of 393 women with cervical cytologic changes confirmed by the Papanicolaou test was undertaken from April 3, 2006, to April 3, 2007. The Hybrid Capture 2 assay was performed. HPV-positive women underwent genotyping for types 16, 18, and 45. Colposcopy and biopsy were performed in 317 (80.7%) and 249 women (63.4%), respectively. The results were analyzed by age groups.

Results. Of all the women with cervical intraepithelial changes, 59% were positive for HR HPV, and 62% were positive for HPV types 16, 18, and 45. HPV types 16, 18, and 45 were detected in 54.8% of women with ASC-US/AGUS/ASC-H, 50.0% of women with LSIL, and 75.6% of women with HSIL. After confirmation of any histological and colposcopic changes, HPV types 16, 18, and 45 were detected in 68.0% and 69.0% of women, respectively. Moreover, 84.2% of the women with HSIL and high-grade colposcopic changes, and 78.5% of the women with HSIL and CIN 2/CIN 2-3/CIN 3/carcinoma in situ were positive for HPV types 16, 18, and 45. The sensitivity of the Papanicolaou test together with the Hybrid Capture 2 test compared with the Papanicolaou test together with the HPV 16/18/45 test diagnosing CIN 2+ changes did not differ (96.7% vs. 97.1%), but the specificity was higher (40.3% vs. 8.0%).

Conclusions. The majority of the cytologic, colposcopic, and histological changes were caused by HPV types 16, 18, and 45. Despite the high prevalence of HPV types 16, 18, and 45, testing for these genotypes together with the Papanicolaou test did not improve the diagnosis of high-grade cervical intraepithelial lesions.

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide (1) with the prevalence of high-risk (HR) HPV in asymptomatic women varying from 2% to 44% (2). Most of HPV infections are transient: 54% resolve spontaneously in one year (3) and 91% in two years (4); however, 10% to 60% of women positive for HPV will have the same genotype one year later (1).

HR HPV DNA in invasive cervical cancer is detected in 75% to 100% of cases (5). Cervical cancer is the third most common cancer in women worldwide and accounts for 9% of all female cancers (6). Other HPV-related cancers, such as anal, vulvar, vaginal, penile, and pharyngeal, both in men and women accounts for additional 0.7% of cancers; therefore, HPV is responsible for 5.2% of all cancers worldwide (7).

The mean morbidity from cervical cancer in Lithuania is twice as big as the mean morbidity in the European Union countries (22 vs. 10.7 cases per 100 000 women) (8). Despite the screening program, the incidence of cervical cancer and mortality from the disease is rising in Lithuania (9). According to the data of the Lithuanian Cancer Registry, the morbidity from cervical cancer is 28 cases per 100 000 women (10). The increasing mortality from cervical cancer among young women aged 15–34 years is being observed (11).

For prevention and early detection of cervical cancer, it is important to detect not only cervical intraepithelial changes, but also to identify the presence of HR HPV and its type as well. If the results of HR HPV test are positive, the possibility of cervical intraepithelial neoplasia (CIN) can be prognosticated even if there are no cytologic changes in the cervix (12–14). The risk of mild cervical changes

Correspondence to K. Jarienė, Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, Eivenių 2, 50028 Kaunas, Lithuania
E-mail: kristej@gmail.com

leading to severe cervical changes (CIN 3) is higher when detecting HR HPV types, especially HPV type 16, compared with lower-risk HPV types (15–17). The possibility for regression of CIN 2 cervical changes caused by HPV type 16 is lower compared with cervical changes caused by other HR HPV types (18). Cervical intraepithelial changes regress when infection with HR HPV resolves spontaneously (19). HR HPV-positive women, even without cytologic changes, have a 210-fold higher risk of developing CIN 3 in 6 years as compared with HR HPV-negative women (20). Only 9 women per 10 000 will have CIN 3 if they are HR HPV-negative with normal cytologic findings (21). HPV type 16 is more likely to persist and is more aggressive compared with other types (22). Though the prevalence of HR HPV has changed recently (because of the added data from East Asia), HPV types 16 and 18 remain most prevalent all over the world (23, 24). HPV types 31, 33, and 45 compete to take the third place in most countries though the increasing prevalence of HPV type 58 has been observed recently (mostly in East Asia) (24). However, HPV types 16, 18, and 45 are the only ones that are more common in invasive forms compared with precancerous lesions (22, 25).

The aim of the study was to determine the prevalence of HR HPV and HPV types 16, 18, and 45 in women with abnormal cervical cytologic changes in relation to colposcopic and histological findings.

Material and Methods

A prospective study of 393 women referred to the Department of Obstetrics and Gynecology, Hospital of Lithuanian University of Health Sciences (HLUHS), from other Lithuanian health care units with suspected abnormal cervical changes detected during the cervical cancer-screening program from April 3, 2006, to April 3, 2007, was carried out. The study was approved by the Bioethics Committee. The diagnoses in medical records from other health care units were based on Papanicolaou tests. The data of 12 women with normal cytologic results and 32 women with uninformative cytologic results were analyzed as well because they had had abnormal results of Papanicolaou tests a few months before (up to half a year), and cervical neoplasia was suspected. Besides, the data of 4 women who were suspected of having carcinoma in situ according to the results of Papanicolaou tests were analyzed. All the women provided informed consent to participate in the study and filled in a questionnaire about risk factors and quality of life. The surveillance and treatment was performed under the abnormal cervical cytology surveillance guidelines approved in HLUHS (colposcopy, direct biopsy, endocervical curettage, or cervical conization were performed according to the indications). Papanicolaou tests were repeated in 377 women (95.9%); colposcopy and biopsy were performed in 317 (80.7%) and 249 women (63.4%), respectively.

Cytologic results were classified as follows: normal findings, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells of undetermined significance (ASC-US)/atypical squamous cells, cannot exclude HSIL (ASC-H)/atypical glandular cells of undetermined significance (AGUS), carcinoma in situ, and noninformative results. Biopsy results were classified as follows: normal findings, endocervicitis, CIN 1/CIN 1-2, CIN 2/CIN 2-3/CIN 3/carcinoma in situ, polypus, and cervical cancer (planocellular or adenocarcinoma). Colposcopic results were classified as follows: normal findings, low-grade, high-grade, various changes (keratosis, erosion, inflammation, polypus, and condyloma), noninformative or unsatisfactory results (the transformation zone was not seen), and not detailed changes (these were the results of not properly evaluated colposcopic findings, but the findings, according to the medical records, could be classified as low-grade or high-grade colposcopic changes). The results were analyzed by age groups: 20–29, 30–39, and 40–49 years. HPV DNA was tested using the Hybrid Capture 2 assay (HC2, Digene Corporation, Gaithersburg, MD 20878 USA) according to the manufacturer's instructions, and results for each specimen were expressed in relative light unit/cut-off (RLU/CO) value. The HC2 test detects 13 HR HPV types; the results of the test were considered positive when the RLU/PC value was more than 1. To detect HPV types 16, 18, and 45 in biological specimens, the HPV 16/18/45 Probe Set was used (Digene Corporation, Gaithersburg, Maryland 20878 USA; Catalog No. 5160–1150). The Probe Set can detect any of these HPV types, but it cannot distinguish among the 3 types. HPV RNA probe sequences are complementary to approximately the complete genome of each virus.

The data were gathered in the database. Statistical analysis was performed by SPSS program, version 16.0. The distribution of abnormal cervical results was determined by evaluating the proportion of abnormal results. To establish relationships, cross tabulation was applied. The chi-square value, degrees of freedom, and P value were calculated. For comparison of two proportions, the z test was employed. The difference was considered statistically significant when $P<0.05$. Sensitivity and specificity of cytology and HPV testing were calculated.

Results

Analysis of the results revealed that 20–29-year-old women made up 22.4% of the study population

(n=88); 30–39-year-old women, 53.1% (n=209); and 40–49-year-old women, 24.5% (n=96). In fact, 177 women (45.0%) who had a Papanicolaou test repeated in HLUHS were positive for HR HPV, and 110 (62.1%) of them were positive for HPV types 16, 18, and 45. There was no significant difference in the prevalence of human papillomavirus comparing all age groups (χ^2=1.56, P=0.5). Of the HR HPV-positive women, 23.2% were in the 20–29-year age group, more than half (55.1%) were in the 30–39-year age group, and 21.7% were in the 40–49-year age group.

Analysis of the women positive for HR HPV showed that 62% were identified as having HPV types 16, 18, and 45. In the 20–29-year age group, of the 43 women, 26 (60.5%) were positive for HPV types 16, 18, and 45. There were 62 women (59.8%) in the 30–39-year age group (n=103) and 29 women (72.5%) in the 40–49-year age group (n=40) who were positive for HPV types 16, 18, and 45 (P>0.05).

The distribution of Papanicolaou test results of women positive for HR HPV and HPV types 16, 18, and 45 according to the age groups is shown in Table 1.

For women who were positive for HR HPV, HSILs were significantly more common in older age groups compared with the youngest one, while ASC-US/AGUS/ASC-H were more common in the youngest age group compared with the older ones (P<0.05). HPV types 16, 18, and 45 were responsible for the greatest part of all HSIL changes in all age groups (from 68.0% to 86.4%).

The distribution of biopsy results among women positive and negative for HPV differed significantly (χ^2=64.94, P=0.0005). Endocervicitis was diagnosed in 18.5% of the HR HPV-positive women compared with 50.0% of the HR HPV-negative women, while CIN 2/CIN 2-3/CIN 3/carcinoma in situ changes were significantly more common in women positive for HPV (63.7% vs. 16.7%). CIN 1/CIN 1-2 histological changes differed neither in HPV-positive women nor HPV-negative women. One of the 4 cervical cancer cases was HPV-negative.

Of the 249 women who had a biopsy performed, 147 (58.9%) were positive for HR HPV, and 100 (67.8%) had HPV types 16, 18, and 45. The distribution of biopsy results of women positive for HPV and for HPV types 16, 18, and 45 is shown in Table 2. The frequency of HR HPV according to biopsy results differed significantly (P<0.05). HPV types 16, 18, and 45 were responsible for the greatest part of any cervical neoplasia: these types were detected in 61.1% of women with CIN 1/CIN 1-2 and 74.2% of women with CIN 2/CIN 2-3/CIN 3/carcinoma in situ. In women positive for HPV 16, 18, and 45, endocervicitis and CIN 1/CIN 1-2 were significantly more common in the youngest age group compared with the older ones.

Histological changes in women positive for HR HPV were not significantly more common in any groups of cytologic changes compared with women negative for HR HPV. HSIL changes were more common among HPV-positive women in CIN 2/CIN 2-3/CIN 3/carcinoma in situ histology group compared with HPV-negative women, though the difference was not significant (Table 3). Endocervicitis with normal cytologic findings was more common among HR HPV-negative women compared with HPV-positive women (P<0.05). ASC-US/AGUS/ASC-H with no histological changes were not significantly but twice as more often observed in HR HPV-positive women compared with HR HPV-negative women, though the number of cases was insufficient to have reliable results. HPV types 16, 18, and 45 in LSIL and HSIL cytologic groups with CIN 2/CIN 2-3/CIN 3/carcinoma in situ histological changes were identified in 66.7% and 78.5% of the women, respectively.

The sensitivity and specificity of the HC2 test for 13 HPV types and the HPV 16/18/45 test in combination with cytologic results for diagnosing CIN 2+ histological changes were calculated.

The sensitivity of the HC2 test and the HPV 16/18/45 test was 83.6% (95% CI, 74.8%–92.5%) and 75.0% (95% CI, 63.7%–86.3%), while the specificity was 63.6% (95% CI, 53.0%–74.2%) and 46.8% (95% CI, 28.6%–65.0%), respectively.

The sensitivity and specificity of the Papanicolaou test in combination with the HC2 test for diagnosing CIN 2+ were 96.7% (95% CI, 92.1%–101.0%) and 40.3% (95% CI, 25.8%–54.7%), respectively. The sensitivity of the Papanicolaou test in combination with the HPV 16/18/45 test for CIN 2+ changes was very similar, i.e., 97.1% (95% CI, 92.2%–102.1%), but the specificity was very low, i.e., 8% (95% CI, 5.6%–21.6%).

The distribution of colposcopic results for HR HPV-positive women and HR HPV-negative women differed significantly (χ^2=26.96, P=0.0005). Severe or not detailed colposcopic changes were significantly more often observed for HR HPV-positive women compared with HR HPV-negative women (26.1% vs. 11.2% and 18.8% vs. 9.2%, respectively), while normal colposcopic results were more often observed for HR HPV-negative women than HR HPV-positive women (32.2% vs. 13.9%). Low-grade changes, various changes, and noninformative results did not differ significantly either for HPV-positive women or for HPV-negative women.

Of the 306 women who had colposcopy performed, 165 (53.9%) were positive for HR HPV, and 109 (66.1%) were positive for HPV types 16, 18, and 45. The major part of low-grade changes

Table 1. Papanicolaou Test Results of Women Positive for Human Papillomavirus and Human Papillomavirus Types 16, 18, and 45 by Age Groups

Age Group	Normal Findings	ASC-US/AGUS/ASC–H	LSIL	HSIL
	HPV(+)/ HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)
20–29 (n=40)	6 (15.0)/2 (33.3)	11 (27.5)**/6 (54.5)	9 (22.5)/3 (33.3)	14 (35.0)/12 (85.7)
30–39 (n=99)	14 (14.3)/6 (42.9)	14 (14.3)/7 (50.0)	20 (20.4)/11 (55.0)	51 (51.0)*/35 (68.0)
40–49 (n=38)	1 (2.6)/0 (0)	6 (15.8)/4 (66.7)	9 (23.7)/5 (55.6)	22 (57.9)*/19 (86.4)
Total (n=177)	21 (11.9)/8 (38.1)	31 (17.6)/17 (54.8)	38 (21.6)/19 (50.0)	87 (48.9)/66 (75.6)

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude HSIL; AGUS, atypical glandular cells of undetermined significance; HVP, human papillomavirus.
*P<0.05, as compard with 20–29-year age group in HSIL group.
**P<0.05, as compared with other age groups in ASC-US/AGUS/ASC-H group.

Table 2. The Distribution of Biopsy Results of Women Positive for Human Papillomavirus and Human Papillomavirus Types 16, 18, and 45 by Age Groups

Age Group	Normal Findings	Endocervicitis	CIN 1/CIN 1-2	CIN 2/CIN 3/ Ca in situ	Polypus	Carcinoma
	HPV(+)/HPV 16, 18, and 45 n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)	HPV(+)/HPV 16, 18, and 45 (+) n (%)
20–29 (n=28)	0	8 (28.6)/ 5 (62.5)**	3 (10.7)/ 3 (100.0)*	17 (60.7)/ 12 (70.6)	0	0
30–39 (n=81)	3 (3.8)/ 3 (100.0)	13 (16.3)/ 5 (38.5)	11 (13.8)/ 6 (54.5)	51 (62.5)/ 37 (72.0)	1 (1.3)/0	2 (2.5)/ 2 (100.0)
40–49 (n=38)	1 (2.6)/ 1 (100.0)	6 (15.8)/ 2 (33.3)	4 (10.5)/ 2 (50.0)	26 (68.4)/ 21 (80.8)	0	1 (2.6)/ 1 (100.0)
Total (n=147)	4 (2.7)/ 4 (100.0)	27 (18.5)/ 12 (44.4)	18 (12.3)/ 11 (61.1)	94 (63.7)/ 70 (74.2)	1 (0.7)/0	3 (2.1)/ 3 (100.0)

CIN, cervical intraepithelial neoplasia; HVP, human papillomavirus.
*P<0.05, 20–29-year age group vs. other age groups in the group with CIN 1/CIN 1-2 histological findings.
**P<0.05, 20–29-year age group vs. other age groups in the group with endocervicitis.

Table 3. The Distribution of Biopsy Results in Relation to the Results of Papanicolaou Test and Human Papillomavirus Test (N=239)

Biopsy	Normal Findings	LSIL	HSIL	ASC-US/AGUS/ ASC–H
	HPV(+)/HPV(–)/HVP 16, 18, and 45 (+) n (%)	HPV(+)/HPV(–)/ HVP 16, 18, and 45 (+) n (%)	HPV(+)/HPV(–)/ HVP 16, 18, and 45 (+) n (%)	HPV(+)/HPV(–)/ HVP 16, 18, and 45 (+) n (%)
Normal findings (n=20)	1 (25.0)/5 (31.3)/ 1 (100.0)	0/4 (25.0)/0	0/1 (6.3)/0	3 (75.0)/6 (37.5)/ 3 (100.0)
Endocervicitis (n=76)	1 (3.7)/17 (34.7)*/ 0	9 (33.3)/10 (20.4)/ 4 (44.4)	5 (18.5)/0/2 (40.0)	12 (44.4)/22 (44.9)/ 6 (50.0)
CIN 1/CIN 1–2 (n=28)	2 (13.3)/6 (46.2)/ 1 (50.0)	6 (40.0)/1 (7.7)/ 2 (33.3)	5 (33.3)/0/5 (100.0)	2 (33.3)/6 (46.2)/ 1 (50.0)
CIN 2/CIN 2–3/CIN 3/ Ca in situ (n=106)	3 (3.4)/3 (17.6)/ 1 (33.3)	12 (13.6)/3 (17.6)/ 8 (66.7)	66 (73.9)/9 (52.9)/ 52 (78.5)	8 (9.1)/2 (11.8)/ 5 (62.5)
Polypus (n=5)	0/1 (25.0)/0	0	1 (100.0)/1 (25.0)/0	0/2 (50.0)/0
Planocellular carcinoma/ adenocarcinoma (n=4)	0	0	3 (100.0)/0/3 (100.0)	0/1 (100.0)/0

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude HSIL; AGUS, atypical glandular cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HVP, human papillomavirus.
*P<0.05, HR HPV-negative vs. HR HPV-positive women.

(64.7%), high-grade changes (81.4%), various changes (60.9%), noninformative results (81.2%), and not detailed results (58.1%) were caused by HPV types 16, 18, and 45. There were 23 HPV-positive women with normal colposcopic results, 47.8% of whom had HPV types 16, 18, and 45.

Colposcopic results in both HPV-positive and HPV-negative women with no cytologic changes did not differ significantly. Compared with HR HPV-negative women, HSILs in HR HPV-positive women were significantly more common in all groups of colposcopic results, except when colposcopic findings were evaluated not properly. Severe colposcopic changes were significantly more common in HR HPV-positive women with HSIL compared with HR HPV-negative women (90.5% vs. 23.5%). In LSIL cases, when benign and severe colposcopic changes were documented, HPV types 16, 18, and 45 were detected in 61.5% and 100%, respectively, and in HSIL cases, the corresponding percentages were 75.0% and 84.2%, respectively.

Discussion

According to our data, the prevalence of HR HPV in age groups did not differ significantly: 60.5%, 59.8%, and 72.5% of the women aged 20–29, 30–39, and 40–49 years, respectively, had HPV types 16, 18, and 45. The incidence of HR HPV was significantly different in the groups of cytologic changes. Nearly two-thirds (62.1%) of the women who had undergone a repeated Papanicolaou test in HLUHS were positive for HPV types 16, 18, and 45. HR HPV types were detected in 11.9% of the women with normal cytologic findings, 38.1% of whom were positive for HPV types 16, 18, and 45. These data correspond to the worldwide data: the estimated global prevalence of HR HPV in women with normal cytologic findings was 11.7% (26), while the prevalence of HPV types 16, 18, and 45 among HPV-positive women with normal cytologic findings was about 36% (2, 23). According to our data, 50.0% of the women positive for HR HPV with LSIL cytologic changes had HPV types 16, 18, and 45. The average percentage worldwide is about 40% (16). Our data indicate that 75.6% of the HR HPV-positive women with HSIL and 54.8% of those with ASC-US/AGUS/ASC-H had HPV types 16, 18, and 45. It has been noticed that although there is no considerable prevalence of HPV type 45 in women with HSIL worldwide, 55% of HPV-positive women with HSIL also had HPV types 16, 18, and 45 (22). There are no meta-analysis data available how HPV is distributed among women with ASC-US. According to various data, HPV types 16 and 18 are responsible for 15% to 32% of borderline changes (27). Our data show that the prevalence of HPV types 16, 18, and 45 in the presence of various abnormal cytologic findings does not correspond to the worldwide data, except for normal cytologic findings. This may be due to the subjectivity of the Papanicolaou test as the result of the Papanicolaou test depends on the experience and skills of the examiner and due to different HPV diagnostic methods.

The first peak of HR HPV prevalence is observed in women in their early 20s up to 25 years (2), but the infection is mostly transient (28, 29). Severe cervical intraepithelial changes are observed in teenagers and women up to 25 years as well, straight after the acquisition of HR HPV infection (30). According to the data reported, the peak incidence of HPV infection occurs within 5 to 10 years after the beginning of the sexual life (31). The prevalence of HPV decreases with age, but persistence increases (32). According to our data, there was no significant difference in the prevalence of HPV in women of different age groups. This may be likely because the present study was focused on the prevalence of HR HPV in women with abnormal cytologic findings, but not in the general population.

Analysis of all the studies done in Europe demonstrated the highest prevalence of HR HPV (29.1%) in Russia (33). The overall prevalence of HPV among women without cancer pathology in Lithuania is 26.7% (34), while the prevalence of HR HPV in the general population is reported to be 25.1% (35). Lithuania has the leading position in both mortality and morbidity of cervical cancer in the Baltic countries. Our data indicated that 11.9% of women with normal cytologic findings were positive for HR HPV, though the total number of the women studied was only 21, and 38.1% of them had HPV types 16, 18, and 45. According to other study carried out in Lithuania, the overall prevalence of HR HPV among women with normal cytologic findings was 21.4%, but HR HPV in this study was detected using PCR general and type-specific HPV primers (34).

HPV type 16 is detected in 2.25% of women in the population (25.5% of all HPV-positive women with normal cytologic findings), and HPV type 18 in 0.76% (7.5% of all HPV-positive women with normal cytologic findings) (23). HPV type 45 takes only the 11th place on the HR HPV list, and its prevalence in women with normal cytologic findings is only 0.4%, but it is one of the 5 most common types detected in invasive cancer cases (2). Cohort studies show that the infection with HPV of types 16 and 18 is more likely to progress compared with HPV of other types (36), while the infection with HR HPV types persist longer than that with low-risk HPV types (37). If HPV genotyping be-

came routinely available, the detection of HPV types 16 and 18 would be useful in clinical management of ASC-US and LSIL with the diagnosis of CIN 2. HR HPV-negative cases (especially HPV 16) could be managed less aggressively through more frequent surveillance rather than immediate treatment, while HR HPV-positive cases that are more likely to become CIN 3 and least regressive should be treated according to the CIN 3 management protocol (18). Our data showed that despite women's age, after the diagnosis of LSIL or ASC-US/AGUS/ASC-H with the histology of CIN2/CIN2-3/CIN3/carcinoma in situ, HPV types 16, 18, and 45 were detected in 66.7% and 62.5% of the subjects studied, respectively. The prevalence of HPV types 16, 18, and 45 in CIN 2/CIN 2-3/CIN 3/carcinoma in situ cases was similar in all age groups: 70.6% in the 20–29-year age group, 72.0% in the 30–39-year age group, and 80.8% in the 40–49-year age group. These data prove once more that genotyping for HPV 16, 18, and 45 is very important in primary screening, especially among women of reproductive age, and could be used in every day practice. However, our data obtained analyzing the sensitivity and specificity of two different tests for diagnosing CIN 2+ changes showed controversial results: the HC2 test was found to be more sensitive and specific compared with the HPV 16/18/45 test. Analysis of all the tests in combination showed that the sensitivity of the HC2 test in combination with the Papanicolaou test for diagnosing CIN 2+ cases compared with the HPV 16/18/45 test in combination with the Papanicolaou test was nearly the same, but the specificity was higher.

HR HPV testing is more sensitive than the Papanicolaou test in ASCUS cases (38). According to the results of meta-analysis done in 2009, 43% of women with ASC-US were positive for HR HPV (15). According to our data, HR HPV was detected in 32.3% of the women with ASC-US/AGUS/ASC-H (38), and 54.8% of them were positive for HPV genotypes 16, 18, and 45, i.e., 50.0% in the 30–39-year age group, 54.5% in the 20–29-year age group, and 66.7% in the 40–49-year age group. According to other study carried out in Lithuania, 23.8% of women with ASCUS/AGUS/ASC-H were positive for HR HPV (34). Consensus guidelines state that HPV DNA testing should be done for every woman with ASCUS (39).

Two-thirds of women with LSIL cytologic changes are found to be positive for HR HPV; therefore, the necessity of HPV testing is limited in such cases. According to the results of meta-analysis done in 2009, 76% of women with LSIL were positive for HR HPV (15). HPV types 16 and 18 in HPV-positive women were found in 35% of all LSIL cases. Our data showed that 55.1% of the women with LSIL were positive for HR HPV (40), and half of them were positive for HPV types 16, 18, and 45, i.e., 33.3% in the 20–29-year age group, 55.0% in the 30–39-year age group, and 55.6% in the 40–49-year age group. According to other study carried out in Lithuania, HR HPV was detected in 46.7% of women with LSIL (34). The risk for CIN 3 in women positive for HPV type 16 with LSIL was 39%, while in other HR HPV-positive (that can be detected by the HC2 method) women with LSIL, the risk for CIN 3 was nearly 4 times lower, i.e., only 10% (36).

A meta-analysis done in 2007 reported that the prevalence of HR HPV in HSIL was 85% (22). The overall prevalence of HPV type 16 and 18 in HSIL cases was 52%. According to our data, 87.8% of HSIL cases were positive for HR HPV (40), and 75.6% of them were positive for HPV types 16, 18, and 45. According to other studies done in Lithuania, HR HPV was detected in 62.1% to 79.3% women with HSIL (10, 34, 41), but the data were analyzed using PCR methods.

According to the worldwide data, the prevalence of HPV types 16 and 18 in women with normal cytologic findings ranges from 32% to 33%. Besides, 50.0% of HSIL cases, 70.0% of invasive cervical cancer cases, and 81.5% of adenocarcinoma cases are positive for these 2 types (42–45). HPV types 16 and 18, compared with other HR HPV types, are more likely to persist and progress (44). The same can be said about HPV type 45. Depending on the country, HPV type 16 is responsible for 50%–60%, HPV type 18 for 10%–20%, and HPV type 45 for 4%–8% of all cervical cancers (23, 25, 46). HPV types 16, 18, and 45 are the only ones more often detected in invasive forms than precancerous lesions (25). This demonstrates the carcinogenicity of these types and the necessity of HPV genotyping. All our cancer cases were identified as having HPV types 16, 18, and 45, but the sample size was too small. Anyway, our data showed that 59% of all the women with abnormal results of Papanicolaou test were positive for HR HPV, and 65% of them were positive for types 16, 18, and 45. According to the results of the other study done in Lithuania, only 45.5% of all the women with the abnormal results of Papanicolaou test were positive for HR HPV, but different methods for HR HPV detection were used (41). As the target-amplification free assay provides a genotyping method for highly specific detection of HPV 16, 18, and 45 without the complexity of PCR technology (47), genotyping for HPV 16, 18, and 45 could be offered in the surveillance of HR HPV-positive women with abnormal cytologic findings, especially in the reproductive age, but the results of sensitivity and specificity of two different

tests in diagnosing CIN 2+ changes lead to more careful evaluation of the disease.

The analysis of all the tests in combination shows that the sensitivity of the HPV test compared with the HPV 16/18/45 test in correlation with the Papanicolaou test is nearly the same, but the specificity differs.

HPV types 16, 18, and 45 were predominant in all cases with any colposcopic findings: e.g., 64.7% in low-grade changes, 81.4% in high-grade changes, and 81.8% when colposcopy was not informative. Moreover, 61.1% of CIN 1/CIN 1-2 cases and 74.2% of CIN 2/CIN 2-3/CIN 3/carcinoma in situ cases were positive for these HPV types.

HPV testing improves the accuracy of colposcopy in the detection of CIN in women with ASC-US and LSIL cytologic changes (48). The comparison and analysis of different cases of cervical pathology, as well as their surveillance and methods of management (cytology, colposcopy, and molecular biology), are rather complicated as there is no unified standardized protocol available (49). Colposcopy does not show a direct association between infection with HPV and CIN changes. Colposcopy is the main method to detect HPV-induced CIN changes. Neither the progression of such changes nor presumable histological results are possible to predict (50). Our data showed that 13.9% of the women positive for HR HPV had normal colposcopic findings. Colposcopic changes (low-grade, high-grade, various changes, and even normal colposcopic results) were more often observed in HPV-negative women with ASC-US/AGUS/ASC-H, though the difference was not significant. There were a great number of not detailed colposcopic results (14.2%), and 58.1% of the women in this group were positive for HPV types 16, 18, and 45. The colposcopic findings in our study should be analyzed with caution because colposcopy was performed not only by specialists. It has been reported that the sensitivity of colposcopy to distinguish normal colposcopic findings from pathological ones is relatively high, but the sensitivity in differentiating low-grade changes from high-grade changes is only about 56% (51).

In LSIL cases, low-grade colposcopic changes, normal colposcopic results, CIN 1/CIN 1-2, or endocervicitis were not significantly more often observed in HR HPV-positive women as compared with HR HPV-negative women. Any colposcopic changes (even normal colposcopic results) were significantly more often documented in HR HPV-positive women with HSIL. And of course, when diagnosing severe cervical changes and cervical cancer, the sensitivity of colposcopy reaches 85%, and the colposcopy is more accurate if, in such cases, the results are combined with the results of the Papanicolaou test (52).

When analyzing the ASC-US/AGUS/ASC-H cytology group, endocervicitis was diagnosed equally for women positive and negative for HR HPV, while CIN 1/CIN 1-2 histology was more often observed in HR HPV-negative women. There can be some reasons for this. Papanicolaou test results frequently show some degree of subjectivity and much depends on the professional competence possessed by the researcher. Atypical cells of undetermined significance, however, can be detected in the cases of bacterial endocervicitis. Though the bacterial changes in the cervix were not investigated, our results do not disprove that: endocervicitis for HR HPV-negative women with normal cytology was significantly more often diagnosed compared with HR HPV-positive women (34.7% vs. 3.7%, $P<0.05$).

When analyzing the associations between cytology, histology, and HR HPV, it was observed that despite any cytologic changes (LSIL, HSIL, or ASC-US/AGUS/ASC-H), biopsy changes were not significantly more common in the women positive for HR HPV compared with the women negative for HR HPV. It means that either histological or cytologic results were inadequately evaluated. The results of both tests should be evaluated only by experienced specialists. The accuracy of biopsy results also depends on the proficiency of the persons who perform it. Biopsy should be performed after the evaluation of colposcopic results (direct biopsy); thus, with the lack of skills while performing colposcopy, the colposcopy might be done and the results might be evaluated inadequately, and biopsy might be taken from the areas without cervical lesions or less-affected areas. On the other hand, evaluation of associations between colposcopy, cytology, and HR HPV results showed that colposcopic changes were significantly more often observed in HR HPV-positive women with HSIL, except inadequately evaluated cases of colposcopy, compared with HR HPV-negative women. Even if biopsy specimens are taken from the site where no colposcopic changes are seen, CIN 2+ changes can be identified in 12.7% to 37.1% of the cases. Therefore, a random biopsy is recommended for all women who have high-grade cytologic changes (53). However, the sensitivity of colposcopy in diagnosing CIN is higher, when 5-biopsy gold standard is utilized (54).

Conclusions

Our study showed that HPV types 16, 18, and 45 were responsible for 62% of cytologic, 66% of colposcopic, and 68% of histological changes, but the HPV 16/18/45 test together with the Papanicolaou test did not improve the diagnosis of high-grade cervical intraepithelial lesions.

Statement of Conflict of Interest

The authors state no conflict of interest.

References

1. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24 Suppl 1:S1-15.
2. de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007;7(7):453-9.
3. Cobo F, Concha A, Ortiz M. Human papillomavirus (HPV) type distribution in females with abnormal cervical cytology. A correlation with histological study. Open Virol J 2009;3:60-6.
4. Safaeian M, Solomon D, Wacholder S, Schiffman M, Castle P. Risk of precancer and follow-up management strategies for women with human papillomavirus-negative atypical squamous cells of undetermined significance. Obstet Gynecol 2007;109(6):1325-31.
5. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87(11):796-802.
6. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.
7. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006;118(12): 3030-44.
8. Arbyn M, Raifu AO, Autier P, Ferlay J. Burden of cervical cancer in Europe: estimates for 2004. Ann Oncol 2007; 18(10):1708-15.
9. Arbyn M, Antoine J, Magi M, Smailyte G, Stengrevics A, Suteu O, et al. Trends in cervical cancer incidence and mortality in the Baltic countries, Bulgaria and Romania. Int J Cancer 2011;128(8):1899-907.
10. Domza G, Gudlevičienė Z, Kazbarienė B, Didžiapetrienė J. Evaluation of human papillomavirus induced cervical intraepithelial lesions risk for progression. Medicinos teorija ir praktika 2008;14(4):346-50.
11. Aleknavičienė B, Smailytė G, Elaawar B, Kurtinaitis J. Gimdos kaklelio vėžys. Sergamumo bei mirtingumo pokyčiai Lietuvoje. (Cervical cancer: recent trends of incidence and mortality in Lithuania.) Medicina (Kaunas) 2002;38(2): 223-30.
12. Kjaer S, Hogdall E, Frederiksen K, Munk C, van den Brule A, Svare E, et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. Cancer Res 2006;66(21):10630-6.
13. Rozendaal L, Westerga J, van der Linden JC, Walboomers JM, Voorhorst FJ, Risse EK, et al. PCR based high risk HPV testing is superior to neural network based screening for predicting incident CIN III in women with normal cytology and borderline changes. J Clin Pathol 2000;53(8):606-11.
14. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95(1):46-52.
15. Arbyn M, Martin-Hirsch P, Buntinx F, Van Ranst M, Paraskevaidis E, Dillner J. Triage of women with equivocal or low-grade cervical cytology results: a meta-analysis of the HPV test positivity rate. J Cell Mol Med 2009;13(4):648-59.
16. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 2005;14(5):1157-64.
17. Schiffman M, Khan MJ, Solomon D, Herrero R, Wacholder S, Hildesheim A, et al. A study of the impact of adding HPV types to cervical cancer screening and triage tests. J Natl Cancer Inst 2005;97(2):147-50.
18. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. Obstet Gynecol 2009;113(1):18-25.
19. Bulkmans NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int J Cancer 2004;110(1):94-101.
20. Rozendaal L, Walboomers JM, van der Linden JC, Voorhorst FJ, Kenemans P, Helmerhorst TJ, et al. PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. Int J Cancer 1996;68(6):766-9.
21. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006;24 Suppl 3: S3/78-89.
22. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121(3):621-32.
23. Bosch FX, de Sanjose S. The epidemiology of human papillomavirus infection and cervical cancer. Dis Markers 2007;23(4):213-27.
24. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. Int J Cancer 2011;128(4):927-35.
25. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003;88(1):63-73.
26. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010;202(12):1789-99.
27. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24 Suppl 3:S3/26-34.
28. Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol 2004;57(1):68-72.
29. Evander M, Edlund K, Gustafsson A, Jonsson M, Karlsson R, Rylander E, et al. Human papillomavirus infection is transient in young women: a population-based cohort study. J Infect Dis 1995;171(4):1026-30.
30. Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357(9271):1831-6.
31. Munoz N, Manalastas R Jr, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24-45 years: a randomised, double-blind trial. Lancet 2009; 373(9679):1949-57.
32. Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis 2005;191(11):1808-16.
33. Alexandrova YN, Lyshchov AA, Safronnikova NR, Imyanitov EN, Hanson KP. Features of HPV infection among the healthy attendants of gynecological practice in St. Petersburg, Russia. Cancer Lett 1999;145(1-2):43-8.
34. Gudlevičienė Z, Didžiapetrienė J, Sužiedelis K, Lapkauskaitė L. Žmogaus papilomos viruso, jo tipų ir variantų tyrimai. (Investigation of human papillomavirus, its types and vari-

ants.) Medicina (Kaunas) 2005;41(11):910-5.
35. Kliučinskas M, Nadišauskienė RJ, Minkauskienė M. Prevalence and risk factors of HPV infection among high-risk rural and urban Lithuanian women. Gynecol Obstet Invest 2006;62(3):173-80.
36. Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6(4):204.
37. Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999;180(5):1415-23.
38. Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet 2003;362(9399):1871-6.
39. Wright TC Jr, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. J Low Genit Tract Dis 2002;6(2):127-43.
40. Jarienė K, Minkauskienė M, Nadišauskienė R, Kliučinskas M, Vaitkienė D. Didelės rizikos žmogaus papilomos viruso įtakoti gimdos kaklelio citologiniai pokyčiai bei sąsajos su histologiniais bei kolposkopiniais radiniais (I dalis). (High risk human papillomavirus and abnormal cervical cytology. Correlations with histologic and colposcopic findings. Part one.) Lietuvos akušerija ir ginekologija 2010;13(1):22-30.
41. Gudlevičienė Z, Šepetienė A, Didžiapetrienė J, Valuckas KP, Smailytė G, Drąsutienė G, et al. Infekuotumas atskirų žmogaus papilomos tipų virusais esant intraepitelinių gimdos kaklelio pokyčių. (Prevalence of human papillomavirus types in cervical intraepithelial lesions.) Medicina (Kaunas) 2010;46(9):616-23.
42. Castellsague X, Diaz M, de Sanjose S, Munoz N, Herrero R, Franceschi S, et al. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. J Natl Cancer Inst 2006;98(5):303-15.
43. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 2003;89(1):101-5.
44. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97(14):1072-9.
45. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(6):518-27.
46. Munoz N, Bosch FX, Castellsague X, Diaz M, de Sanjose S, Hammouda D, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111(2):278-85.
47. Thai H, Rangwala S, Gay T, Keating K, McLeod S, Nazarenko I, et al. An HPV 16, 18, and 45 genotyping test based on Hybrid Capture technology. J Clin Virol 2009;45 Suppl 1:S93-7.
48. Monsonego J, Pintos J, Semaille C, Beumont M, Dachez R, Zerat L, et al. Human papillomavirus testing improves the accuracy of colposcopy in detection of cervical intraepithelial neoplasia. Int J Gynecol Cancer 2006;16(2):591-8.
49. Nirchio V, Lipsi R, Fusilli S, Ciccone E, Murino L, Santangelo A, et al. HPV infection: comparison between morphological studies and molecular biology [In Italian]. Pathologica 2008;100(3):149-55.
50. Ji HX, Yliskoski M, Vayrynen M, Hippelainen M, Syrjanen S, Syrjanen K. Colposcopic analysis of genital human papillomavirus infections during an 8-year prospective follow-up. Int J Gynaecol Obstet 1991;36(4):291-300.
51. Louwers JA, Kocken M, ter Harmsel WA, Verheijen RH. Digital colposcopy: ready for use? An overview of literature. BJOG 2009;116(2):220-9.
52. Benedet JL, Matisic JP, Bertrand MA. An analysis of 84244 patients from the British Columbia cytology-colposcopy program. Gynecol Oncol 2004;92(1):127-34.
53. Nam K, Chung S, Kwak J, Cha S, Kim J, Jeon S, et al. Random biopsy after colposcopy-directed biopsy improves the diagnosis of cervical intraepithelial neoplasia grade 2 or worse. J Low Genit Tract Dis 2010;14(4):346-51.
54. Pretorius RG, Bao YP, Belinson JL, Burchette RJ, Smith JS, Qiao YL. Inappropriate gold standard bias in cervical cancer screening studies. Int J Cancer 2007;121(10):2218-24.

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