

Prevalence of human papillomavirus types in cervical intraepithelial lesions

Živilė Gudlevičienė¹, Agnė Šepetienė^{2, 3}, Janina Didžiapetrienė^{1, 4}, Konstantinas Povilas Valuckas¹, Giedrė Smalytė¹, Gražina Drąsutienė², Rūta Jolanta Nadišauskienė⁵

¹Institute of Oncology, Vilnius University, ²Clinic of Obstetrics and Gynecology, Faculty of Medicine, Vilnius University, ³Vilnius University Hospital Santariškių Klinikos, ⁴Clinic of Internal Medicine, General Practice and Oncology, Faculty of Medicine, Vilnius University, ⁵Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, Lithuania

Key words: cervical intraepithelial lesions; prevalence of human papillomavirus types.

Summary. Background. Since the implementation of the cervical cancer screening program in Lithuania in 2004, cervical cancer incidence rates have stabilized during a 4-year period: in 2006 and 2007, 508 and 485 new cases, respectively, were diagnosed. Human papillomavirus (HPV) infection is one of the main risk factors for cervical cancer and development of intraepithelial lesions. However, not only HPV, but also HPV type, is a very important factor for malignant transformation. Cervical intraepithelial lesions with HPV 16 and 18 more frequently progress to cancer. To date, in Lithuania, studies only on HPV prevalence and risk factors have been carried out, and less attention has been paid to the identification of HPV types. The aim of this study was to identify the most common HPV types in women with various cytological lesions.

Material and methods. A total of 246 women with various cytological lesions (atypical squamous cells of undetermined significance [ASCUS], low-grade squamous intraepithelial lesion [LSIL], and high-grade squamous intraepithelial lesion [HSIL]) were included into the study. All the women were screened for HPV infections followed by HPV typing for types 6, 11, 16, 18, 31, 33, 45, and 59. Polymerase chain reaction was used.

Results. Less than half (45.5%) of women with cytological lesions were infected with HPV. The highest prevalence of HPV was detected in women with HSILs (62.1%) and CIN2 (86.7%). HPV typing revealed that the most frequent type was HPV 16 (64.3%); HPV 18 and HPV 33 accounted for 5.4% and 4.5% of cases, respectively. Based on cytologic diagnosis, HPV 16 was more frequently found in women with HSILs than women with ASCUS (77.8% vs. 50.0%).

Conclusions. The prevalence of HPV infection in women with cytological lesions was 45.5%. The highest prevalence of HPV was detected in women with HSILs (cytologic investigation) and CIN2 (histologic investigation). HPV 16 is the most common type in women with various cervical intraepithelial lesions.

Introduction

Morbidity and mortality rates for cervical cancer in Lithuania have been increasing during the last decades and have even exceeded the mean respective European rates. It is noteworthy that the mean morbidity rates for cervical cancer in the European Union countries are 11.9 cases per 100 000 female population (1). The rise in the morbidity rates for cervical cancer in Lithuania has begun since 1992: in 1998–1999, 17.4 cases were registered per 100 000 women, and in 2004, the figure reached 31.1 cases. After the initiation of the cervical cancer screening program in 2004, the morbidity rates stabilized in 2008, and even decreasing trends were observed. In 2005, 500

new cases were diagnosed (standardized index, 27.4); in 2006, 508 (standardized index, 28.0); and in 2007, 485 new cases (standardized index, 26.9) (2). It is noteworthy that in the second half of 2004, at the initiation of the cervical cancer screening program, more cases of precancerous cervical pathologies were diagnosed than before. Human papillomavirus (HPV) undoubtedly plays a key role as the main risk factor in cervical carcinogenesis (3–5). On a global scale, HPV is detected in nearly 100% of cervical cancers, while in healthy women without intraepithelial lesions in the cervix, HPV infections are detected, on the average, in 10.4% of cases (6). However, the prevalence of HPV infection varies across different geographic

Correspondence to Ž. Gudlevičienė, Institute of Oncology, Vilnius University, P. Baublio 3b, 08406 Vilnius, Lithuania
E-mail: zivile.gudleviciene@gmail.com

Adresas susirašinėti: Ž. Gudlevičienė, VU Onkologijos institutas, P. Baublio 3b, 08406 Vilnius
El. paštas: zivile.gudleviciene@gmail.com

regions. HPV is one of the most prevalent sexually transmitted infections. Around 630 million people worldwide are infected with HPV; moreover, even 75–80% of sexually active people become infected with HPV at some point in their lives (7). Even though the prevalence of different HPV types varies across different geographic regions, types 16 and 18 are most frequently detected. HPV type 16 is most common and is detected in approximately 50% to 55% of all cases of cervical cancer. This virus type, like HPV types 18 and 45, plays an important role in infection becoming persisting and in causing cell transformation (6, 8). The highest prevalence of HPV infection is found among young women, and they are infected most frequently with various types of viruses. A spontaneous and sudden drop in the prevalence of the infection is found in the group of middle-aged women, and a second rise is observed in the postmenopausal age group (5). Intraepithelial lesions in the cervix begin to develop several years after the actual infection, i.e., in middle-aged women, and progression to cervical cancer is slow (2–12 years), which allows for early diagnosis and treatment. The progression of intraepithelial lesions to cancer is affected not only by the presence of the infection per se, but also by particular virus types. In the presence of highly oncogenic HPV type 16 or 18 infection, the progression is more rapid than in cases of infections with other HPV types (e.g., 31, 33, etc.) (9–11). Thus, HPV infection and oncogenic types of HPV are important risk factors for cervical cancer, yet they are not the only risk factors. The women's age, the affected area in the cervix, and other cancer risk factors (e.g., smoking) are also important for determining the risk of progression in intraepithelial lesions. Previous studies have shown that cancer usually develops when the CIN3-affected area involves not only the superficial epithelium, but the endocervical glands as well (12, 13). New sensitive molecular testing techniques allow for the evaluation of the progression in lesions using multiple molecular markers, such as the amount of the virus in the organism, HPV *E2* gene integration, viral activity markers (HPV mRNA), gene (e.g., *p16*) expression or DNA methylation changes (14), etc. The aim of our study was to determine the prevalence of HPV infection among women and to identify the types of HPV in cervical intraepithelial lesions of various degrees.

Material and methods

Patient inclusion and examination

The study included 246 18–70-year-old women who during primary examination had already been diagnosed with cervical pathology (the regular PAP smear contained atypical squamous cells of undetermined significance [ASCUS], atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion [ASC–

H], low-grade squamous intraepithelial lesion [LSIL], or high-grade squamous intraepithelial lesion [HSIL]). The women were invited for a second visit to the Consultation Polyclinic of Vilnius University Hospital Santariškių Klinikos (the examination period was from March 1, 2008, to July 31, 2008). Women who agreed to participate in the study signed the invitation to the study and the informed consent form. The study protocol, the invitation to the study, and the informed consent form were approved by the Lithuanian Bioethics Committee (permission for a biomedical study No. 15, granted on February 29, 2008). Pregnant women were excluded from the study. During the visit to the gynecologist, the women underwent gynecologic examination, colposcopy, and biopsy (if required according to the care algorithms for women with cervical intraepithelial lesions), as well as sampling for HPV testing. Women who were found to have marked inflammatory or atrophic alterations in the cervix during the gynecologic examination received anti-inflammatory treatment or local hormone therapy with estrogens. After the treatment, all women underwent repeated cytologic examination (PAP test) at the Institute of Oncology, Vilnius University; by applying polymerase chain reaction (PCR), the presence of HPV infection was checked, and types of HPV were identified.

Detection of HPV and identification of its types (6, 11, 16, 18, 31, 33, 45, and 59)

Material for the detection of HPV infection (epithelial cells scraped off the cervix) was obtained by using a sterile brush and was then placed into 1 mL of PBS buffer solution. Subsequently, DNA was isolated from the samples in laboratory settings, and HPV testing was performed. The studied DNA was isolated from nonrefrigerated samples and was stored at temperature of -20°C .

Samples for HPV typing were prepared in several stages. First, DNA was isolated. This procedure was performed using a commercial *Sorpoclean* DNA extraction module (joint-stock company (JSC) "SORPO diagnostics," Lithuania); DNA was isolated following the manufacturer's recommendations.

PCR technique. PCR for HPV detection was conducted using 50 μL of mixture consisting of 45- μL commercial *HPV Master Mix* (JSC "SORPO diagnostics," Lithuania) and 5 μL of the studied DNA. PCR was performed following the manufacturer's recommendations. Before performing PCR for HPV detection, the isolated DNA was tested to determine whether all samples contained the β -globin gene (15). Each PCR for HPV detection was performed using a positive and negative control. For the positive control, SiHa and HeLa

cells were used, while the negative control consisted of samples without DNA (containing deionized water).

HPV-positive samples underwent further testing: several PCR procedures for HPV typing, using commercial *HPV 16,18 Master Mix*; *HPV 31, 33, 59 Master Mix*; and *HPV 6, 11, 45 Master Mix* kits (JSC "SORPO diagnostics," Lithuania). PCR was performed following the manufacturer's recommendations.

If three PCR procedures failed to identify the HPV type, the sample was tested for the presence of infection with high-risk (HR) and low-risk (LR) HPV type groups. For this purpose, commercial *Seeplex HPV4 ACE Screening* kit (Seegene Inc., Korea) was used; the HR HPV types were 35, 51, 56, 58, 66, 67, and 70, and the LR HPV types were 42, 43, and 44 (16). The kit is convenient to use because one reaction may identify infection with HR or LR virus groups, infection with type 16 and 18 viruses, and also provides internal and external sample control.

Visualization of PCR products using electrophoresis. Amplified PCR products were analyzed using electrophoresis. Electrophoresis was performed in 2% agarose gel stained with ethidium bromide. Following electrophoresis, ethidium bromide-stained PCR products were analyzed in a transilluminator using a UV light source (320 nm).

Statistical analysis

Data analysis was performed using statistical analysis software package SPSS (version 15.0, SPSS Inc. Chicago, IL, USA). The subjects of the study were analyzed according to their age, diagnosed cervical intraepithelial lesions (the cytologic diagnosis), the presence of HPV infection, and HPV types. Differences between the groups were evaluated using the χ^2 (chi-square) or Fisher's exact test. Differences were considered statistically significant if the level of significance was $P < 0.05$.

Results

A total of 246 women were included into the study between March 1, 2008, and July 31, 2008. The subjects underwent cytologic examination and were diagnosed with cervical intraepithelial lesions of various degrees (ranging from ASCUS to HSIL). The mean age was 37.9 years (from 18 to 70 years; SD, 10.53). All women were distributed into 5 age groups. The distribution of the studied women by age is presented in Fig. 1.

Results of testing for the presence of HPV infection

HPV testing using PCR showed that 45.5% of women (112 out of 246) were infected. The evaluation of the

Table 1. The prevalence of human papillomavirus (HPV) infection among women with diagnosed cervical intraepithelial lesions by age groups

Age group in years	HPV infection				Total, n
	negative		positive		
	n	%	n	%	
<25	11	33.3	22	66.7	33
26–35	30	40.5	44	59.5	74
36–45	42	61.8	26	38.2	68
46–55	46	75.4	15	24.6	61
>56	5	50.0	5	50.0	10
Total	134	54.5	112	45.5	246

Table 2. The prevalence of human papillomavirus (HPV) infection among women according to cytologic diagnosis

Cytologic finding	HPV infection				Total, n
	negative		positive		
	n	%	n	%	
AGC	1	50.0	1	50.0	2
ASC-H	5	83.3	1	16.7	6
ASCUS	70	65.4	37	34.6	107
LSIL	25	56.8	19	43.2	44
HSIL	33	37.9	54	62.1	87
Total	134	54.5	112	45.5	246

AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance. Differences between the groups were evaluated using Fisher's exact test ($\chi^2=45.41$, $P=0.00006$).

prevalence of HPV infection in different age groups showed that HPV was most common among young women aged up to 25 years (accounting for 66.7% of cases). The prevalence of HPV infection decreased with age and was the lowest among women aged 46–55 years (24.6%). The second rise in the prevalence of HPV infection was observed in women older than 50 years; however, only 10 women of such age participated in our study (Table 1).

Further analysis showed that the highest prevalence of HPV infection was among women in whom cytologic examination revealed HSIL (62.1%), while the prevalence of HPV in case of ASCUS was nearly half of that observed in the presence of HSIL (34.6%) (Table 2). The evaluation of differences between the groups revealed statistically significant differences in the prevalence of HPV with relation to the cytologic diagnosis: the prevalence of HPV was the lowest in the presence of minor lesions (ASCUS), increased with more severe lesions (LSIL), and was the highest in the presence of HSIL ($P=0.00006$).

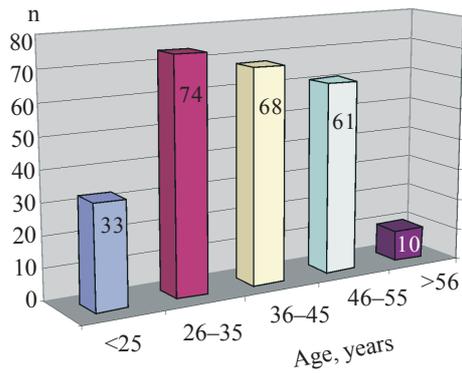


Fig. 1. Distribution of the examined women by age (mean, 37.9; min, 18; max, 70; SD, 10.53)

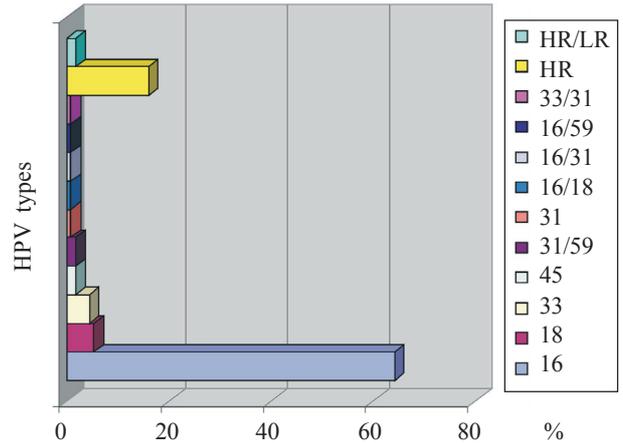
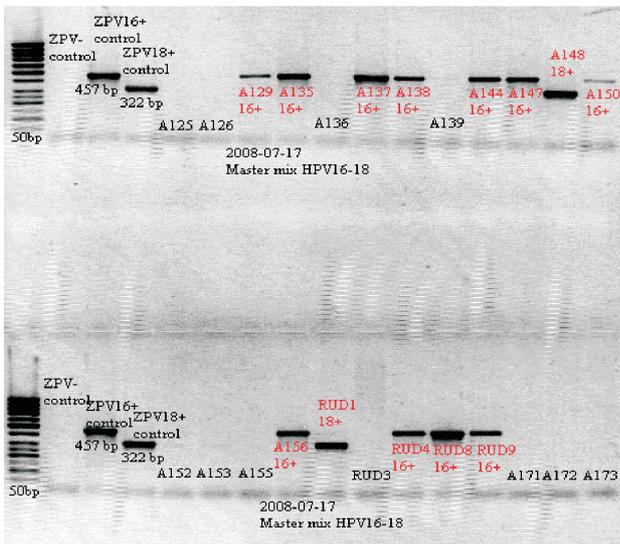
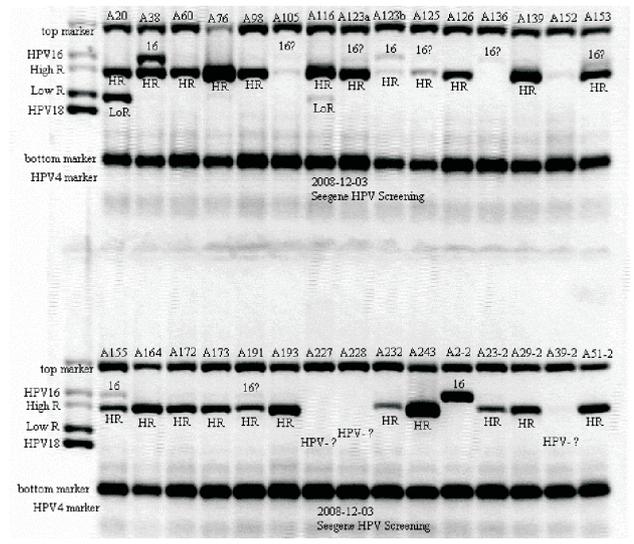


Fig. 2. The prevalence of various types of human papillomavirus (HPV) infection among the examined women



HPV-positive samples are indicated in red



HR, high-risk HPV types; Low R, low-risk HPV types; 16, HPV type 16

Fig. 3. Human papillomavirus (HPV) typing and visualization using electrophoresis

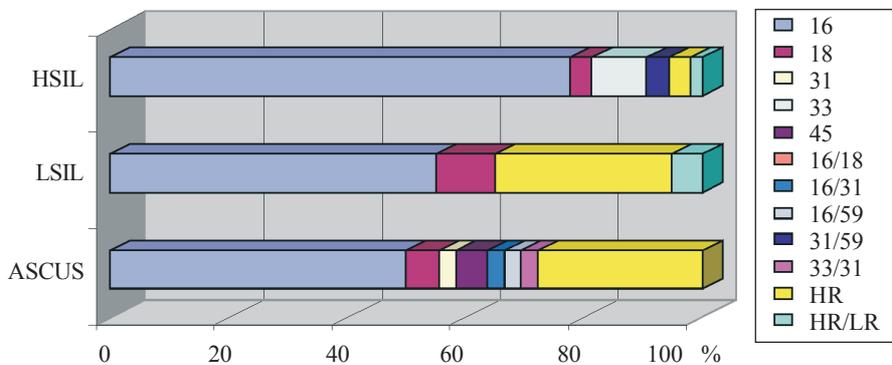


Fig. 4. The prevalence of various types of human papillomavirus infection among the examined women according to the cytological diagnosis HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; HR, high-risk human papillomavirus types; LR, low-risk human papillomavirus types

Results of HPV typing

HPV typing showed that the majority (64.3%) of the women were infected with HPV type 16. Even 75 women were infected with this type of virus, and three of them had a double infection, i.e., with HPV types 16/18, 16/31, or 16/59. Only approximately 5% of women were diagnosed with type 18 (5.4%) and type 33 (4.5%) infection. Concerning other HPV types, in individual cases, infections with types 31, 45, and 59 or double infections with HPV types 31/59 and 31/33 were detected (Fig. 2). None of the samples were infected with HPV types 6 and 11. If the identification of virus type during PCR with specific primers for HPV types 16, 18, 31, 45, and 59 failed, additional CR procedures were performed to determine the presence of high-risk or low-risk HPV groups (using the kit *Seeplex HPV4 ACE Screening*, Seegene Ltd., Korea). The majority of the samples (16.1%) were infected with high-risk (highly carcinogenic) HPV. Two women were diagnosed with low-risk HPV infection; it is noteworthy that they also had coinfection with high-risk group viruses as well. Fig. 3 presents images showing HPV typing using two different kits. On the left, the *SORPO diagnostics* kit was used for the identification of HPV types 16 and 18, and on the right, the *Seegene* kit was applied to differentiate viruses into high-risk and low-risk groups, and also to identify HPV types 16 and 18.

The analysis of the findings according to the subjects' age showed that the prevalence of HPV type 16 infection was similar in nearly all age groups (between 60.0% and 68.2%), although it was somewhat lower (68.2%) among young women (up to 25 years of age), compared to that among older subjects (60.0%).

Cytologically, HPV type 16 was most frequently detected in females with diagnosed HSIL (77.8%), while in those with ASCUS, this type of virus was less common (50.0%) (Fig. 4).

It is noteworthy that none of the examined women were diagnosed with low-risk HPV type 6 or 11 infections, although 2 women were diagnosed with double infections – both low cancer risk and high cancer risk HPV types.

Discussion

HPV infection is one of the major risk factors of cervical cancer and precancerous pathology (17). Epidemiological studies have shown that the majority of women (even up to 80%) are infected with HPV at least once in their lives (18). An extensive study on the prevalence of HPV was performed by the Catalan Institute of Oncology (CIO), and the findings of this extensive meta-analysis were published in 2007 by the World Health Organization (WHO) (19). The meta-

analysis included data from studies performed between 1999–2005, involving a total of 157 897 women without any cytologic changes detected in PAP smears. The analysis showed that DNA of HPV was identified in the cervical samples of 10.4% (95% CI, 10.2–10.7) of these women. The results of this analysis also showed that higher prevalence of HPV was more characteristic of economically underdeveloped countries (13.4%; 95% CI, 13.1–13.7), compared to that in economically developed countries (8.4; 95% CI, 8.3–8.6). On a global level, it has been determined that this infection is most prevalent among African women (22.1%; 95% CI, 20.9–23.4), especially in Eastern Africa (31.6%; 95% CI, 29.5–33.8), while the lowest prevalence is observed among South-East Asian women (6.2%; 95% CI, 5.5–7.0). Similar results were obtained in a study conducted by the International Agency of Research on Cancer (IARC). They examined 15 613 15–74-year-old women from 11 countries and performed a general meta-analysis. This study has certain advantages because the same protocol for sample selection and risk factor evaluation was applied in all countries. Testing of samples for the presence of HPV infection was carried out in a centralized laboratory, using PCR with common primers for HPV detection – GP5+/6+. The established age-standardized prevalence of HPV DNA was 10.5% (95% CI, 9.9–11.0) (20). More information on HPV studies is available on the WHO website www.who.int/hpvcentre/en/.

In Lithuania, HPV studies on the role of this infection in the carcinogenesis of cervical cancer were initiated around 1999. Kliučinskas et al. analyzed factors affecting the prevalence and persistence of HPV and their association with cervical intraepithelial lesions (21, 22). The study included healthy women applying to a gynecologist for a preventive checkup. The examination did not involve identification of particular virus types, but rather focused on detecting the presence of infection with high-risk viruses, using the Hybrid Capture 2 technique. The study showed that the prevalence of infection with high-risk viruses among these women was 25.1%. The study also showed that women living in urban areas were more commonly infected with this virus, compared to those residing in rural areas (27.0 vs. 11.1%, $P < 0.05$). In 2004–2005, the Institute of Oncology, Vilnius University, conducted a study on the role of HPV and its types and variants in the risk of cervical cancer (23, 24). The study revealed a high prevalence of HPV infection among Lithuanian women with cervical cancer; the prevalence of HPV infection among such women was 92.0%, compared with 23.6% among healthy women ($P < 0.0001$). This study involved virus

typing as well. However, it is noteworthy that only a small proportion of the examined women were diagnosed with cervical intraepithelial lesions. Studies performed in Lithuania showed a significantly higher prevalence of HPV, compared to mean global or European rates (25), and thus the morbidity and mortality rates for cervical cancer were higher as well, compared with mean European rates.

Reduction of cervical cancer-related morbidity and mortality requires the development and expansion of screening programs for cervical pathology. It has been ascertained that such programs are ineffective or of limited efficiency in cases of cervical adenocarcinomas (26). It is noteworthy that the new primary prevention measure – HPV vaccine that protects mostly against HPV type 16 and 18 infection – is sufficiently effective for the prevention of this histologic form of cancer. Vaccination of girls and young women is already being applied in some countries, although there still are ongoing discussions regarding which age groups of girls and women should be vaccinated and whether vaccination of boys is expedient. However, studies have shown that the vaccine is most effective when it is applied before the actual infection with HPV types targeted by the vaccine, and thus vaccination of girls and young women should be given priority (27, 28). Thus, the role of HPV studies in screening programs and in the presence of precancerous pathology becomes even more important. It has been established that the prevalence of HPV infection is significantly higher in the presence of pre-malignant changes of various degrees, and this prevalence is related to the degree of changes in intraepithelial lesions. Various studies have shown that the prevalence of HPV infection may be from 50% in the presence of ASCUS and more than 80% in the presence of HSIL, especially in cases when HSIL is confirmed histologically, and CIN2 or CIN3 is detected (6). The results of the studies have also shown that women with varying degrees of intraepithelial alterations and those with no cytologically detected intraepithelial changes demonstrate lower incidence of HPV infection (around the age of 35), and a second rise is observed at older age (around the age of 50) (29). Our study showed that 45.5% of women were infected with HPV in the presence of cervical intraepithelial lesions of various degrees (ranging from ASCUS to HSIL). The highest prevalence of HPV was observed in young women up to 25 years of age (66.7% of cases). Thus, these results also suggest that Lithuanian women with cytologic changes in the cervical mucosa are more commonly infected with HPV, compared with the mean global rates. However, some authors indicate that in the presence of HSIL, which most frequently progresses to cancer, and

in the absence of treatment, even up to 85.0% of women may be infected with HPV (30).

To investigate the prevalence of HPV types over the world, a meta-analysis including 6978 women was conducted and it was found that HPV type 16 was most common in the presence of HSIL. The prevalence of this type of virus on a global scale ranges from 51.8% (95% CI, 50.1–53.5) in Europe to 33.3% (95% CI, 20.4–48.4) in Oceania; the overall prevalence of this HPV type in the presence of HSIL is estimated to be 45.4%. Variations in the prevalence of other types of this virus are not marked and mostly depend on the geographic zone. Even though there are slight variations in the prevalence of HPV in various geographic regions, HPV types 16, 18, 33, 45, 31, 58, 52, and 35 are most commonly detected (30, 31). On the one hand, our findings (the majority of the examined women were infected with HPV types 16 [64.3%], 18 [5.4%], and 33 [4.5%]) corroborate data presented by other authors; on the other hand, the prevalence of HPV infection found in our study exceeds the mean global or European rates.

In most countries, cytologic examination of the cervix, i.e., PAP smear, is the principal technique in cervical cancer screening. However, this technique is also known to have certain disadvantages. In addition to poor sensitivity, which reaches 50–60% in cases of CIN 2/3, the results of the test may also be affected by subjective factors such as faulty sampling and preparation of the smear (5% to 10%) or incorrect laboratory interpretation, which may result in false-positive diagnosis of precancerous conditions and, consequently, it is associated with overtreatment.

The sensitivity of HPV DNA test is higher compared with cytologic testing (66% to 100% in HPV DNA vs. 44% to 78% in cytologic testing), but this test has a lower specificity (61% to 96%) compared to that of cytologic examination (91% to 96%) (32). For this reason, liquid-based techniques are recommended for screening, where the same material could be used for cytologic and HPV or other possible molecular marker testing.

Laboratory testing for HPV infection is recommended in the following cases:

- Unclear PAP test results: when ASCUS is detected or LSIL is suspected;
- In monitoring of women with positive PAP smear (i.e., ASCUS, LSIL, or HSIL), but without confirmed pathology during colposcopy or biopsy;
- In monitoring of women and prognostication of CIN treatment outcomes;
- Alone or in combination with cytologic examination for primary screening in women aged more than 30 years (32).

In addition to that, as mentioned before, cytologic screening alone is not very effective in diagnosing adenocarcinoma – a tumor that is considered more aggressive and characterized by more rapid metastasis and poorer prognosis, compared with squamous cell carcinoma (33). Adenocarcinoma cells are hard to reach with a brush used in taking PAP smears. In such cases, the presence of HPV type 18 suggests adenocarcinoma.

HPV typing is recommended and applied after obtaining positive screening results; mostly, the most common high-risk types (16 and 18) and other HPV types are identified (32). These tests are important in designing new polyvalent vaccines that in the future would protect not only against types 16 and 18, but against other HPV types as well (32). Thus, as mentioned before, HPV testing is more sensitive than cytologic examination or colposcopy. In 2006, the American Society for Colposcopy and Cervical Pathology recommended the application of the HPV DNA test in cervical cancer screening as well. A combination of PAP

smear, HPV DNA test, and biological HPV markers may help to identify HPV-infected women without clinical signs but at high risk of cervical cancer, to select rational treatment for cervical intraepithelial lesions, and to improve the efficacy of cervical cancer screening and prevention. There is currently an ongoing search for new markers of virus activity or integration (e.g., mRNA or E2 gene deletion) that would help to identify women at risk of progression of intraepithelial lesions.

Conclusions

Our study showed that 45.5% of the examined women with cervical intraepithelial lesions were infected with human papillomavirus; the highest prevalence of human papillomavirus infection with predominant type 16 was observed in cases of high-grade squamous intraepithelial lesions.

Acknowledgments

We would like to thank the State Studies Foundation for their support (agreement No. T-44/08).

Infekuotumas atskirų žmogaus papilomos tipų virusais esant intraepitelinių gimdos kaklelio pokyčių

Živilė Gudlevičienė¹, Agnė Šepetienė^{2, 3}, Janina Didžiapetrienė^{1, 4}, Konstantinas Povilas Valuckas¹, Giedrė Smailytė¹, Gražina Drąsutienė², Rūta Jolanta Nadišauskienė⁵

¹Vilniaus universiteto Onkologijos institutas, ²Vilniaus universiteto Medicinos fakulteto Akušerijos ir ginekologijos klinika,

³Vilniaus universiteto ligoninės Santariškių klinika, ⁴Vilniaus universiteto Medicinos fakulteto Vidaus ligų, šeimos medicinos ir onkologijos klinika, ⁵Lietuvos sveikatos mokslų universiteto Medicinos akademijos Akušerijos ir ginekologijos klinika

Raktažodžiai: intraepiteliniai gimdos kaklelio pokyčiai, infekuotumas atskirų tipų ŽPV.

Santrauka. Įvadas. Lietuvoje 2004 m. pradėjus vykdyti moterų patikros dėl gimdos kaklelio patologijos programą, 2008 m. sergamumo gimdos kaklelio vėžiu rodikliai stabilizavosi. 2006 m. diagnozuoti 508 nauji atvejai, 2007 m. – 485. ŽPV infekcija yra svarbus ir gimdos kaklelio vėžio, ir ikivėžinės patologijos rizikos veiksnys. Formuojantis vėžiui, didelę įtaką turi ne tik infekuotumas virusu, bet ir konkretūs viruso tipai. Esant infekuotumui 16 ar 18 tipų virusais, ikivėžiniai pokyčiai progresuoja greičiau. Iki šiol Lietuvoje buvo atliekami ŽPV paplitimo ir rizikos veiksnių tyrimai, o mažiau dėmesio skiriama ŽPV tipų identifikavimui. Šio tyrimo tikslas – nustatyti infekuotumą ŽPV ir identifikuoti jo tipus esant įvairaus laipsnio intraepitelinių gimdos kaklelio pokyčių.

Tyrimo medžiaga ir metodai. Į tyrimą įtrauktos 246 moterys, kurioms citologinio tyrimo metu nustatyta įvairaus laipsnio intraepitelinių gimdos kaklelio pokyčių (ASCUS, LSIL, HSIL). Visoms moterims polimerazės grandininės reakcijos (PGR) metodu buvo nustatyta, kurio tipo – 6, 11, 16, 18, 31, 33, 45, 59 ŽPV jos infekuotos.

Rezultatai. Atlikus infekuotumo ŽPV tyrimus, nustatyta, kad, esant gimdos kaklelio citologinių pokyčių, ŽPV infekuota 45,5 proc. moterų. Didžiausias infekuotumas ŽPV tų moterų, kurioms citologinio tyrimo metu diagnozuota HSIL (62,1 proc.), o histologinio tyrimo metu – CIN2 (86,7 proc.). Atlikus ŽPV tipų identifikavimo tyrimus, nustatyta, kad daugiausia moterų buvo infekuotos 16 tipo (64,3 proc.), mažiau – 18 tipo (5,4 proc.) ir 33 tipo ŽPV (4,5 proc.). Remiantis citologine diagnoze, 16 tipo ŽPV dažniausiai identifikuotas moterims, esant HSIL (77,8 proc.), o esant ASCUS, šio tipo virusas rastas rečiau (50,0 proc.).

Išvada. ŽPV infekuota 45,5 proc. moterų, kurioms nustatyta intraepitelinių gimdos kaklelio pokyčių; didžiausias infekuotumas ŽPV, vyraujant 16 jo tipui, yra HSIL (citologinis tyrimas) ir CIN2 (histologinis tyrimas) atveju.

References

1. Castellsagué X, de Sanjose S, Aguado T, Louie KS, Bruni L, Muñoz J, et al. HPV and cervical cancer in the world. 2007 Report. *Vaccine* 2007;25 (Suppl 3):C1-26.
2. Lietuvos vėžio registras. (Lithuanian Cancer Registry.) Available from: URL: <http://www.vuoi.lt/index.php?1146970586>
3. Hoory T, Monie A, Gravitt P, Wu TC. Molecular epidemiology of human papillomavirus. *J Formos Med Assoc* 2008;107(3):198-217.
4. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiol Mol Biol Rev* 2004;68(2):362-72.
5. Monsonego J, Bosch FX, Coursaget P, Cox JT, Franco E, Frazer I, et al. Cervical cancer control, priorities and new directions [EUROGIN 2003 conclusions]. *Int J Cancer* 2004;108(3):329-33.
6. Bosch FX, Burchell AN, Schiffman M, Giuliano MR, de Sanjose S, Bruni L, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* 2008;26(Suppl 10):K1-16.
7. Medeiros LR, Hilgert JB, Zanini RR, Berwanger O, Bozzetti MC, Mylius LC. Vertical transmission of human papillomavirus: a systematic quantitative review. *Cad Saude Publica* 2005;21(4):1006-15.
8. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. *Infect Dis Obstet Gynecol* 2006;Suppl:40470.
9. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM; ALTS Group. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *J Infect Dis* 2007;195(11):1582-9.
10. Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. *J Natl Cancer Inst* 2005;97(14):1066-71.
11. 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.
12. Ostör AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993;12(2):186-92.
13. Al-Nafussi AI, Hughes DE. Histological features of CIN3 and their value in predicting invasive microinvasive squamous carcinoma. *J Clin Pathol* 1994;47(9):799-804.
14. Gravitt PE, Coutlée F, Iftner T, Sellers JW, Quint WG, Wheeler CM. New technologies in cervical cancer screening. *Vaccine* 2008;26(Suppl 10):K42-52.
15. Ireng LM, Robert A, Gala JL. Quantitative assessment of human β -globin gene expression in vitro by TaqMan real-time reverse transcription-PCR: comparison with competitive reverse transcription-PCR and application to mutations or deletions in noncoding regions. *Clin Chem* 2005;51:2395-6.
16. Manual for multiplex-PCR System for the screening of human papilloma virus (HPV). Available from: URL: http://www.seegene.co.kr/en/download/manual1_hpv4ace.pdf.
17. IARC Monographs on the evaluation of carcinogenic risks to humans. Human papillomaviruses. Vol. 90. Lyon: World Health Organization International Agency for Research on Cancer; 2007.
18. Castellsagué X. Natural history and epidemiology of HPV infection and cervical cancer. *Gynecol Oncol* 2008;110(3 Suppl 2):S4-7.
19. de Sanjose S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz 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.
20. Clifford GM, Gallus S, Herrero R, Muñoz N, Snijders PJ, Vaccarella S, et al.; IARC HPV Prevalence Surveys Study Group. 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(9490):991-8.
21. Kliučinskas M, Nadišauskienė RJ, Padaiga Ž, Spukaitė T. Žmogaus papiloma viruso paplitimas tarp 18-35 metų Kauno moterų. (Prevalence of human papillomavirus among 18-35-year-old Kaunas women.) *Lietuvos akušerija ir ginekologija* 1999;2(1):19-22.
22. 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.
23. Gudlevičienė Ž, Didžiapetrienė J, Sužiedėlis K, Lapkauskaitė L. Žmogaus papilomos viruso, jo tipų ir variantų tyrimai. (Investigation of human papillomavirus, its types and variants.) *Medicina (Kaunas)* 2005;41(11):910-5.
24. Gudlevičienė Ž, Ramael M, Didžiapetrienė J, Uleckienė S, Valuckas KP. Human papillomavirus and p53 polymorphism in Lithuanian cervical carcinoma patients. *Oncology Gynecology* 2006;102:530-3.
25. Maucort-Boulch D, Franceschi S, Plummer M; IARC HPV Prevalence surveys study group. International correlation between human papillomavirus prevalence and cervical cancer incidence. *Cancer Epidemiol Biomarkers Prev* 2008;17(3):717-20.
26. Myers E, Huh WK, Wright JD, Smith JS. The current and future role of screening in the era of HPV vaccination. *Gynecol Oncol* 2008;109(Suppl 2):S31-9.
27. Wright TC, Van Damme P, Schmitt HJ, Meheus A. Chapter 14: HPV vaccine introduction in industrialized countries. *Vaccine* 2006;24(Suppl 3):S3/122-31.
28. Schiller JT, Castellsagué X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. *Vaccine* 2008;26(Suppl 10):K53-61.
29. Franceschi S, Herrero R, Clifford GM, Snijders PJ, Arslan A, Anh PT, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. *Int J Cancer* 2006;119(11):2677-84.
30. 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.
31. 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.
32. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine* 2008;26(Suppl 10):K29-41.
33. Hildesheim A, Hadjimichael O, Schwartz PE, Wheeler CM, Barnes W, Lowell DM, et al. Risk factors for rapid-onset cervical cancer. *Am J Obstet Gynecol* 1999;180(3 Pt 1):571-7.

Received 8 September 2009, accepted 6 September 2010
 Straipsnis gautas 2009 09 08, priimtas 2010 09 06