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ORIGINAL ARTICLE

Association of human papillomavirus infection with cytology, colposcopy, histopathology, and risk factors in the development of low and high-grade lesions of the cervix

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Summary

Purpose: *Persistent human papilloma virus (HPV) infection is fundamental for the development of intraepithelial neoplasia and cervical cancer. The aim of this study was to examine the association between HPV infection and cervical cytology, colposcopy, biopsy and the risk factors entailed.*

Methods: *The study included 784 patients tested by in situ hybridization for HPV type 6/11, 16/18 and 31/33/35 infection. The participants were divided into three categories according to the presence of high squamous intraepithelial lesions (H-SIL), low squamous intraepithelial lesions (L-SIL) and benign histologic findings.*

Results: *Patients that had L-SIL and H-SIL demonstrated a significantly higher percentage of HPV infection than patients with benign histologic findings. The percentage of H-SIL was highest in patients infected with high risk (HR)-HPV types 16/18, who had sexual intercourse before the age of 16 years, with two to five sexual partners.*

Conclusion: *Among high-grade intraepithelial neoplasia, there is a high prevalence of HR-HPV types 16/18, which is associated with the number of sexual partners and early sexual activity. There was a significant association between the presence of abnormal cytology and HR-HPV 16/18 in both groups (H-SIL and L-SIL). Benign colposcopic findings were not found in any patient with H-SIL, where no patient had only one sexual partner.*

Key words: *cervix, HPV infection, precancerous intraepithelial lesions*

Introduction

Cervical cancer is the third most common cancer type in women worldwide [1]. Persistent infection of HPV is the strongest, although not sufficient, factor associated with intraepithelial neoplasia and cervical cancer [2]. The presence of high-risk HPV types in epithelial cells leads to changes that significantly increase the chance of intraepithelial carcinoma [3]. HR-HPV types 16 and 18 cause 70% of all invasive cervical carcinomas [4], 41-67% of high grade squamous intraepithelial lesions (H-SIL), and 16-32% of L-SIL [5]. Low-risk (LR) HPV 6 and 11 genotypes are responsible for the occurrence of genital warts in 80-90% of the cases [6], and are also responsible for the development of L-SIL cervical changes [7]. HPV is the most common sexually transmitted infection (STIs), although most of the changes remain low grade for two years [8,9].

Persistent detection of HR-HPV types is a strong predictor of development of high-grade cervical changes and invasive carcinoma. Infection with HR-HPV results in complex cellular abnormalities and is an important precursor for carcinogenesis and the development of cervical carcinoma. That is why cytology is the most common diagnostic method and the gold standard for screening programs, although its sensitivity in detecting intraepithelial lesions or the stage of disease is

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limited. In a histopathologically confirmed stage of disease, the percentage of false negative cytodiagnosis is significant. Colposcopy, another method for the detection of cervical neoplasia, is characterized by high sensitivity in the detection of an earlier stage of disease. For verification and determination of pathological changes, in addition to the HPV testing, colposcopy and cytologic examination, it is necessary to carry out a biopsy and histopathologic verification [10,11].

The aim of this retrospective study was to examine the association between HPV infection and cervical cytology, colposcopy and biopsy in order to define possible risk factors.

Methods

Patients

In the Clinic of Obstetrics and Gynecology "Narodni front", 784 patients aged 17 to 54, were tested for HPV infection during 2009-2013. The patients gave consent to participate in the study. The research was conducted according to the latest version of the Declaration of Helsinki guidelines and the legal requirements and recommendations of the National and International Institutions related to this scientific research. The study was approved by the Hospital's Ethics Committee. Patients were divided into three groups: the experimental group I consisted of 195 patients with histopathologically confirmed L-SIL changes of the cervix; the experimental group II had 21 patients with histopathologically confirmed H-SIL; the group III (control group) consisted of 569 patients with benign lesions. Cytological findings were classified according to Papanicolaou or cervical cytology Bethesda system from 2001. Colposcopic findings were classified according to International Classification of Cervical Pathology and Colposcopy (IFCPC), Barcelona, 2002. Squamous intraepithelial lesions included: atypical squamous cells of undetermined significance (ASCUS), squamous intraepithelial lesions (SIL), which included pre-cancerous conditions, divided into L-SIL and H-SIL.

Methods

Patients, who had previously undergone colposcopy and cytology (Papanicolaou) and cervical biopsies, with histopathologic verification, gave information concerning age, the number of sexual partners, age at first intercourse, number of births and abortions. The patients were tested for presence of virus types 6/11, 16/18, and 31/33/35 by DNA *in situ* hybridization.

Statistics

Analyses were performed using x^2 test and binary logistic regression to determine the association between the presence of H-SIL, L-SIL and oncogenic HPV types.

The individual impact of oncogenic HPVs, their combined effect (HPV6/11, HPV16/18, HPV 31/33/51) - Model 1 - and the influence of other factors such as age, number of sexual partners and first sexual intercourse before the age of 16 - Model 2- was determined. The age and number of sexual partners entered as continuous variables, while HPV type 6/11 (0 - yes, 1 - negative), HPV type 16/18 (0-positive, 1 - negative), HPV type 31/33/51 (0 - positive, 1 - negative) and the first sexual intercourse before 16 years of age (0 - positive, 1 - negative) entered as random variables. For each odds ratio (OR) there was an estimation of the probability and 95% confidence interval (95% CI). Also, Receiver Operating Characteristics (ROC) curves were constructed, for the proposed regression models and a comparison was made on their predictive value on the basis of the area under the curve (AUC). The various features of the model were classified with respect to AUC values as low ($0.5 \leq AUC < 0.7$), acceptable ($0.7 \leq AUC < 0.8$), good ($0.8 \leq AUC < 0.9$) or excellent ($AUC \geq 0.9$).

Statistical analyses were performed using STATGRAFICH Plus (version 4.2) and CBstat (version 4.3.2) software. All statistical tests were considered significant with a probability of 0.05.

Results

Table 1 indicates the general characteristics of the study groups according to presence of precancerous lesions and HPV typing. The groups diagnosed with H-SIL or L-SIL had a significantly higher percentage of HPV positive findings,when compared to the group with benign lesions ($p<0.001$). In both L-SIL and H-SIL Pap smears were positive in high percentages (64.44 and 71.42%, respectively). A significant percentage of patients, who had two to five sexual partners until the time of examination, were positive to L-SIL, H-SIL, and also with benign lesions. The greatest percentage of patients with H-SIL had sex before the age of 16 years. In patients with normal colposcopy there was no H-SIL, but 14.9% of them had L-SIL. In patients with abnormal colposcopy, 27.5% had L-SIL, but 3.8% of them had H-SIL ; 68.7% of these patients had pathologically benign lesions (Table 1).

No positive HPV 16/18 was found in the control group, while in the one with precancerous lesions, 119 out of 215 (55.34%) patients were found positive to these types of virus. The HPV 31/33/51 were positive in 73 out of 215 (33.95%) patients with precancerous lesions, and it was also positive in 20 (3.51%) patients with cervicitis chronica. The HPV 6/11 were positive in 31 of 215

Table 1. Classification and main characteristics of the study groups

Parameters		*L-SIL N =194 (24.70%) N (%)*	*H-SIL N=21 (2.70%) N (%)*	*Benign findings N=569 (72.60%) N (%)*	*p value*
Age, years, mean±SD		34.7 ± 9.4	37.2 ± 6.1	34.4 ± 9.8	0.371
HPV 6/11	Positive	30 (15.5)	1 (4.8)	3 (0.5)	<0.001
	Negative	164 (84.5)	20 (95.2)	566 (99.5)	
HPV 16/18	Positive	98 (50.5)	21 (100)	0 (0)	<0.001
	Negative	96 (49.5)	0 (0)	569 (100)	
HPV 31/33/51	Positive	68 (35.1)	5 (23.8)	20 (3.5)	<0.001
	Negative	126 (64.9)	16 (76.2)	549 (96.5)	
Pap smear	Normal	69 (35.56)	6 (28.56)	495 (87.0)	<0.001
	Abnormal	125 (64.44)	15 (71.42)	74 (13.0)	
Sexual partners	One	14 (7.2)	0 (0)	52(9.1)	<0.001
	2-5	138 (71.1)	12 (57.1)	471 (82.8)	
	6 and more	42 (21.6)	9 (42.9)	46 (8.1)	
Sex before the age of 16 years	Before	22 (11.3)	6 (28.6)	24 (4.2)	<0.001
	After	172 (88.7)	15 (71.4)	545 (95.8)	
Colposcopy	Normal	11 (14.9)	0 (0)	63 (85.1)	0.002
	Abnormal	154 (27.5)	21 (3.8)	384 (68.7)	

For abbreviations see text

Table 2. Logistic regression analysis for the association of HPV type of viral infection, age, number of sexual partners and sex before the age of 16, with precancerous lesions occurrence

Models		*p value*	*OR*	*95% CI for OR*	
				Lower	*Upper*
HPV 6/11		<0.001	31.81	9.6	105.1
HPV 16/18		<0.001	704.10	97.2	5099.5
HPV 31/33/51		<0.001	14.11	8.32	23.9
Model 1	HPV 6/11	<0.001	173.65	47.2	638.5
	HPV 16/18	<0.001	1465.57	336.3	6386.4
	HPV 31/33/51	<0.001	70.39	35.2	140.6
Model 2	HPV 6/11	<0.001	335.01	79.5	1411.7
	HPV 16/18	<0.001	2773.49	564.1	13635.5
	HPV 31/33/51	<0.001	140.20	58.3	336.9
	Sexual partners	0.088	1.83	0.91	3.66
	Age	<0.001	1.09	1.04	1.14
	Sex before the age of 16 years	0.236	2.22	0.59	8.26

OR: odds ratio, CI: confidence interval, HPV: humanpapilloma virus

Table 3. The results of ROC analysis for discriminating patients with high risk from patients with low risk for precancerous lesions occurrences

Test result variables	*AUC*	*SE*	*p value*	*Asymptotic 95% CI*	
				Lower bound	*Upper bound*
HPV 6/11	0.569	0.024	0.003	0.522	0.617
HPV 16/18	0.777	0.022	<0.001	0.733	0.820
HPV 31/33/51	0.652	0.024	<0.001	0.605	0.699
Model 1	0.947	0.010	<0.001	0.928	0.961
Model 2	0.967	0.07	<0.001	0.952	0.978

CI: confidence interval, AUC: area under the curve, SE: standard error

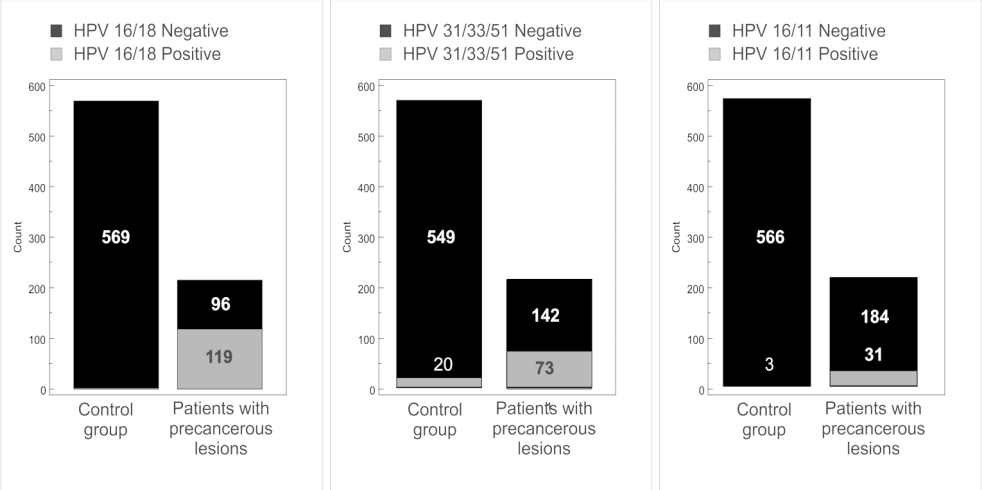

Figure 1. HPV findings in patients diagnosed with high-grade (H-SIL) or low-grade squamous intraepithelial lesions (L-SIL) and in the control group.

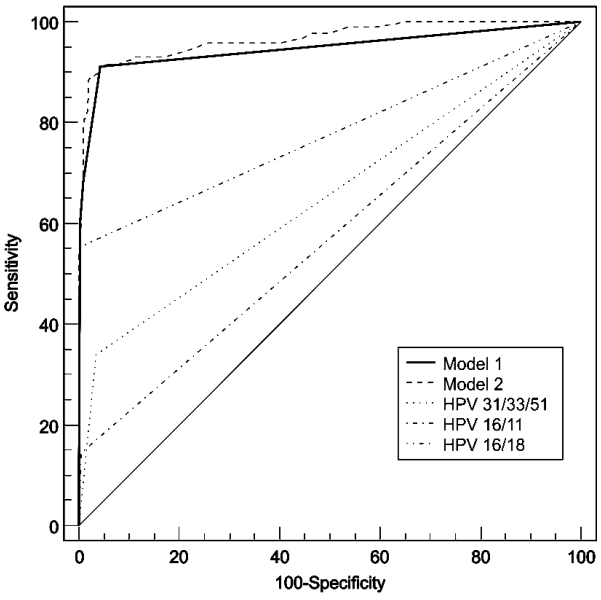

Figure 2. Comparison of ROCs of single HPV assessments, Model 1 and Model 2, for discriminating patients with high risk for precancerous lesions occurrences from patients with low risk.

(14.41%) patients with precancerous lesions, and in 3 (0.52%) patients with cervicitis chronica (Figure 1). Pap smear was 100% and 85.1 % positive in patients with H-SIL and L-SIL, respectively.

A binary logistic regression model was used to investigate the predictors for precancerous lesions occurrence in patients with different types of HPV infection (Table 2). Each of the HPV types proved to be good predictor for precancerous conditions (odds ratios 31.81, 704.10, 14.11; p<0.001), but taken together, these factors had significantly greater predictive value (Model 1). In Model 2, besides the HPV typing, some other factors such as age, sexual partners and sex before the age of 16, were added. Besides the HPV type, the age (odds ratio 1.09, p<0.001), but not sexual partners and sex before the age of 16, was an important predictor for precancerous lesions occurrence.

We also investigated the potential benefit of simultaneous testing for all three types of HPV viruses (HPV 6/11, HPV 6/18, HPV 31/33/51). To achieve this we constructed ROC curves with predictive probabilities and calculated AUCs (Figure 2). The calculated AUC (0.777) for the single HPV 16/18 testing indicated that the clinical accuracy of the applied test was acceptable, contrary to the other two tests (HPV 6/11, HPV 31/33/51). We also constructed ROC curves with predictive probabilities from Model 1 and Model 2. The simultaneous testing for all three types of HPV viruses increased the AUC value. When compared to single tests pair-wise of ROC curves, this improvement was statistically significant (p<0.001) (Table 3).

Discussion

HPV is the most common sexually transmitted infectious agent and a high-risk factor for development of cervical carcinoma and its cytologic precursors. In our study, there were no statistically significant differences between groups with respect to age. Otherwise, the age range of our patients coincided with the age group in which the probability of precancerous lesions occurence dominates [12]. Significantly higher LR-HPV type 6/11 was found in patients with H-SIL and L-SIL changes in the cervix, than in the control group.

The L-SIL changes do not progress to invasive cancer and low grade abnormalities also occur in uninfected women [13]. The mechanism of carcinogenesis of many HPV types is not known. In a large study [12], in less than 1%, the LR-HPV 6 was the main cause of cervical cancer, indicating that more research is needed to clarify the conditions under which these HPV types can cause cancer.

HPV 16 was the most common HR-HPV type found in the cervix and cervical cancer, detected in 50% of patients who were infected, and who developed H-SIL [14]. Early detection of HR-HPV type 16, which is associated with H-SIL, plays an important role in reducing morbidity and mortality from cervical cancer [15]. Other HPV types that were commonly detected in cervical carcinomas include types 18,31, and 45, which are more frequently diagnosed with invasive cervical cancer rather than in precancerous lesions [5,15], suggesting that infection with HR-HPV types 16/18 leads to the highest risk for developing high grade cervical lesions, compared to any other HPV type infection.

The HR-HPV 18 occurs more frequently in adenocarcinoma in situ (ACIS), while in patients with H-SIL, the HR-HPV type 31, or combined infections of HPV 16, 18, 45, and 31, are more frequent [16]. In our study, HR-HPV types 31/33/51 were significantly more in the experimental groups, but very few in the control group. HPV infections are usually transient, especially among young women, while only a small proportion of persistent infection can lead to cervical cancer.

Many studies revealed that sexual behavior may have an impact on the acquisition and development of the infection [17,18]. In our study patients who had only one partner had H-SIL, while only few patients with one partner exhibited L-SIL. This finding suggests that a monogamous lifestyle can reduce the risk of this type of infection and consequently the appearance of precancerous lesions. The small percentage of patients who have had only one partner also indicates that this lifestyle has a relatively small number of women [18]. We found a strong association between the first sexual experience at a younger age and HPV infection, which is consistent with previous studies [19,20]. It has been suggested that getting HPV infection is probably due to the immaturity of the structural transition zone epithelial cervix in adolescents, making it susceptible for entry and persistence of the virus. In contrast to the results of our research, the study of Louvanto et al. [21] demonstrated that with a higher number of sexual partners and sexual activity starting before the age of 13, there is a lower risk of HPV infection [22]. It seems that women who later have sex are at increased risk of infection just because of the fact that younger women, who had longer sexual activity, acquired immunity by the age of 20 years. The same analogy can be applied to the number of sexual partners, which in this study was inversely related to the risk for HPV infection, because a smaller number of partners leads to increased risk. It seems that women who have had many partners before they were included in this study had a lower risk of infection with the same HPV types [22].

Because of their simplicity and the relatively low cost of cytology and HPV testing, these tests are the basis of diagnostic and screening programs around the world [23-25]. In our H-SIL group, less than a third of HPV positive women had normal cytology and H-SIL, while in L-SIL women about 35% of had normal cytology and L-SIL. Normal cytology was found in most of the control group patients.

Cytological diagnosis, colposcopy and other basic methods for the detection of cervical neoplasia are characterized by high sensitivity and ability to detect the initial stages of disease. Strategies in diagnosis, using two positive PCR tests for HPV, had higher sensitivity and specificity than colposcopy [25]. In our study, normal colposcopic results were found in about only 15% of women who were HPV positive and had L-SIL changes on the cervix, and in 85% of the controls with no SIL. In women who have had HPV infection and H-SIL, there were no normal colposcopic results. An important finding was that 27.5% of patients had L-SIL and abnormal colposcopy, while 3.8% of patients had H-SIL and abnormal colposcopy. These results indicate the importance of the essential colposcopy in the diagnosis of early stage disease.

It is well known that HPV infection is the main cause for the development of precancerous lesions of the cervix. Risk factors for HPV infection suggest the importance of getting the demographic, socio-economic, sexual activity and lifestyle information. Based on logistic regression analysis, we found that the HPV 16/18 types are the best predictors of the origin of SILs. Model 1 which is applied to the testing of HPV 16/18, 31/33/35 HPV and HPV 6/11, shows even greater diagnostic reliability than the 0947 AUC (area under the curve). When the same approach was

applied to Model 2, the patient emerged as an independent predictor of cervical disease. These findings suggest that testing for multiple HPV types can improve the diagnosis of precancerous lesions of the cervix.

In conclusion, in high-grade intraepithelial neoplasia, HR-HPV types 16/18 are highly present and this is associated with the number of sexual partners and early sexual activity. Significant presence of these two genotypes was associated with low-grade intraepithelial neoplasia. An important association between the presence of abnormal cytology and HR-HPV 16/18 in both groups (H-SIL and L-SIL) was found. Benign colposcopic findings were not evident in any patient with H-SIL, where no patient had only one partner.

Conflict of interests

The authors declare no confict of interests.

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