Int J Clin Exp Pathol 2017;10(2):1817-1824
www.ijcep.com /ISSN:1936-2625/IJCEP0039690

Original Article

High risk factors associated with HPV persistence after loop electrosurgical excision procedure in patients with intraepithelial neoplasia

Yunyun Yu*, Lingfei Han*, Wen Yu, Yankang Duan, Zhewei Wang, Huiyan Hu, Junwei Zhao, Suman Singh, Fanfei Kong, Lin Jin, Jing Sun, Fang Li

*Department of Gynecology, Cervical Disease Centre, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China. *Equal contributors.*

Received September 8, 2016; Accepted November 20, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Objective: Persistent infection with carcinogenic human papillomavirus (HPV) causes cervical intraepithelial neoplasia (CIN) which, is commonly treated using the loop electrical excision procedure (LEEP). We investigated the factors that associate with the persistency of HPV after LEEP in patients with CIN. Methods: We retrospectively analyzed 860 CIN patients who underwent LEEP. All patients had high-risk HPV (HR-HPV) testing using Hybrid Capture II system. HPV persistency, defined as HPV DNA being present before and 6 months after LEEP, was evaluated. Cervical tissues specimens were collected at the time of LEEP. CD8+ and CD138+ expression were detected by immunohistochemistry and IFN-γ expression was detected by RT-PCR. Results: The HPV persistency rate was 33.3% (286/860). Univariate analysis showed that HPV clearance rate after LEEP in the patients with CIN was higher when patients were younger (Rs=-0.109, P=0.011), lesions with HSIL (Rs=0.141, P<0.001), lesions involving the glands (X^2=12.145, P<0.001) and contraception been used (P=0.001). Persistent HPV infection was more likely to occur when the pretreatment viral load was high (RLU/positive control >100, P=0.027). Patients with persistent HR-HPV infection had lower numbers of CD8+ cells (P=0.022), higher numbers of CD138+ cells (P=0.035) and lower levels of IFN-γ. Conclusion: Age, LSIL, condom contraception used and local immune status are significantly related to HR-HPV persistency after LEEP.

Keywords: Cervical intraepithelial neoplasia, persistent human papillomavirus infection, loop electrosurgical excision procedure

Introduction

Worldwide, cervical cancer is the second most common cancer in women and one of the most severe female health problems [1]. Globally, 520,000 new cases of cervical cancer and 260,000 deaths occur annually [2]. Cervical intraepithelial neoplasia (CIN) is a group of precancerous lesions that is closely related to cervical cancer [3]. If not treated, women with high-grade CIN are at very high risk of developing cervical cancer [4]. Persistent infection with high risk human papillomavirus (HR-HPV) is the foremost cause of cervical cancer and postoperative recurrence [2, 3, 5]. Organized screening involving cytological or HPV testing is very useful for reducing or preventing cervical cancer and post operation recurrence [1].

CIN is a well-known precursor of invasive cervical cancer. The loop electrosurgical excision procedure (LEEP) is frequently used to treat CIN [6-8]. However, recurrence/residual rates range from 5% to 30% [8], and persistent HR-HPV infection is a significant prognostic indicator of recurrence/residual disease after LEEP [9-12]. Age, HPV viral load, margin involvement, and cytology grade are risk factors for recurrence/residual after successful LEEP [4, 13-16].

Studies have also shown the association of local immunity with patient clinical presentations. T-lymphocytes play an important role in humoral and cellular immunity. CD4+ and CD8+ T-cells, which recognize HPV early proteins, have been found among cervical tumor infiltrat-

Table 1. Patient demographics (n=860)

		Number of patients	%
Cytology	Normal	231	26.9
	ASCUS	268	31.2
	ASC-H	80	9.3
	LSIL	185	21.5
	HSIL	96	11.2
Biopsy pathology	LSIL	445	51.7
	HSIL	415	48.3
HPV DNA	Negative (0-1)	101	11.7
	<100	323	37.6
	100-500	182	21.3
	>500	254	29.4
LEEP pathology	LSIL	402	46.7
	HSIL	421	49.0
	CA	9	1.0
	CC	28	3.3
HPV DNA after LEEP	Positive	318	37.0
	Negative	542	63.0

ASCUS: Atypical squamous cells of undetermined significance; ASC-H: Atypical squamous cells-high grade; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesions; CIN: Cervical intraepithelial neoplasia; HPV: Human papillomavirus; RLU/PC: Relative light units/positive control; CC: Chronic cervicitis; and CA: Carcinoma.

ing lymphocytes and are present in the blood of healthy donors and although to a different extent, in patients with CIN and cervical cancer. CD4+ helper T-cell scan directs the body to fight against the virus. There are two subsets of these cells, Th1 and Th2. Th1 cells arouse cellular immunity by activating cytotoxic CD8+ T-cells and secreting interferon-gamma (IFN-γ) [17, 18]. Th2 cells help B-lymphocytes synthesize and secrete antibodies [19, 20]. IFN-γ is known to inhibit HPV oncogene transcription and inhibit the growth of cell lines harboring the viral genome [21, 22]. CD138 (syndecan-1) is a transmembrane sulfuric acid heparin proteoglycan family member. It is a cell adhesion molecule, promoting cell proliferation, cell matrix and cell-cell adhesion by the sulfuric acid-acetic acid heparin chain. CD138 expression in some tumors is obviously missing, which leads to the loss of cell growth and contact inhibition function, which makes the tumor cell proliferation, and highly invasive activity [23]. The local immune response might affect the HPV persist infection [24]. However, studies on the immune modulation of HPV persistence infection after LEEP are limited.

In this study, we assessed the rate and high risk factors of HPV persistence after LEEP in CIN patients, with consideration of HPV viral loads, age, severity of disease, lesions involving the glands, contraception used and local immunity.

Material and methods

Study population

Eight hundred and sixty patients underwent LEEP conization due to CIN at Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, between February 2014 and July 2015 were included in this study. This study was approved by the local Scientific and Ethical Committee (ref: K51533). All samples and data were collected with written consents from patients. Patient demographics, HR-HPV test data, pathology reports, and follow-up data were reviewed from medical records.

HR-HPV titer was detected by HCII (Digene, Maryland, USA) before LEEP and 6 months after LEEP according to the manufacturer's instructions.

Cytology

The cervical cytology in this study was liquid-based (Thinprep cytologic test, TCT, Hologic, USA). Exfoliated cervical cells were collected using a special cytobrush. All specimens were stained using the Papanicolaou method and were evaluated using the Bethesda System. Cytology was divided into negative for intraepithelial lesion (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells with high grade (ASC-H), low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs). For the pathological diagnosis, colposcopy with direct biopsy was performed. The results of the colposcopic-directed biopsy were also divided into two groups: low-grade squamous intraepithelial lesions (LSILs), including Koilocyte, warts and CINI and high-grade squamous intraepithelial lesions (HSILs), including CINII/III and carcinoma in situ (CIS).

Reverse transcription-polymerase chain reaction

Exfoliated cervical cells were collected before LEEP using an endocervical cytobrush and stored in the saline solution. RNA was extracted from cells using Trizol reagent according to the manufacturer's protocol. The concentration

Table 2. Viral Clearance 6 months after LEEP [(n (%)]

	N (viral clearance)	Clearance rate (%)	Rs/X^2	*P*
Age				
21-30	62	66.0 (62/94)		
31-40	249	69.2 (249/360)		
41-50	125	68.3 (125/183)		
51-60	83	56.5 (83/147)		
>60	23	30.3 (23/76)		
Total	542	63.0 (542/860)	Rs=-0.109	0.011
Virus titer before LEEP (RLU/PC)				
<100	263	81.4 (263/323)		
100-500	125	68.7 (125/182)		
>500	152	59.8 (152/254)		
Total	542	71.4 (542/759)	Rs=0.101	0.002
LEEP Pathology				
LSIL	230	57.2 (230/402)		
HSIL	306	72.7 (306/421)		
CA	6	66.7 (6/9)		
Total	542	65.1 (542/832)	Rs=0.141	<0.001
Margin				
Positive	175	58.9 (175/297)		
Negative	367	65.2 (367/563)		
Total	542	63.0 (542/860)	X^2=3.27	0.07
Glands				
Involved	249	70.7 (249/352)		
Not involved	293	57.7 (293/508)		
Total	542	63.0 (542/860)	X^2=15.22	<0.001
Condom contraceptive				
Yes	210	73.2 (210/287)		
No	332	57.9 (332/573)		
Total	542	63.0 (542/860)	X^2=19.03	<0.001

of RNA was determined using SmartSpec plus (Bio-Rad, USA). RNA was reverse transcribed to cDNA using the PrimeScriptTMRT reagent Kit (code: DRR037s, 100 reactions, TaKaRa) according to the manufacturer's protocol. PCR was performed in a 25 μl reaction volume containing 1 μl of cDNA, 12.5 μl of premix Taq (code: D331, 500 μl, TaKaRa), 0.5 μl of forward primer, 0.5 μl of reverse primer and 10.5 μl of dH_2O. The primer sequences for IFN-γ were as follows: Forward 5'-ATGAAATATACAAGTTATATCTTGGCT-3', Reverse 5'-GCGACAGTTCAGCCATCACTTG-3' (420 bp); β-actin: Forward 5'-ATGGGTCAGAAGGATTCCTATGTG-3', Reverse 5'-CTTCATGAGGTGTCAGTCAGGTC-3' (434 bp).

The amplification conditions were as following: preheating at 95°C for 2 min, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 min, and extension at 72°C for 1 min were performed. The PCR products were separated by electrophoresis on a 2% agarose gel and visualized with an Eagle Eye analyzer [25].

Immunohistochemistry

Specimens were taken from resected tissues from CIN patients who underwent LEEP. Paraffin sections were prepared using established techniques [26]. After dewaxing, the paraffin sections were microwaved three times in citric acid (BOSTER) for 6 min. The sections were incubated with H_2O_2 (BOSTER, #AR1108) and 5% BSA (BOSTER), followed by incubation at 4°C overnight using primary monoclonal antibodies against CD8 (clone c8/144B, 1:25; DAKO, Glostrup, Denmark) and CD138 (clone MI15, 1:25; DAKO, Glostrup, Denmark). The paraffin sections were then incubated with HRP-

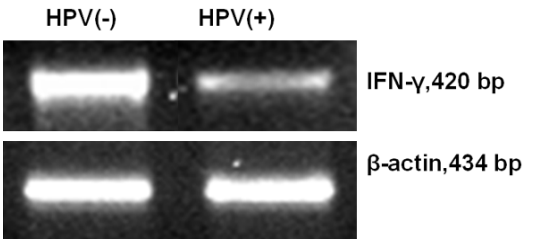

Figure 1. The level of IFN-γ expression was lower in patients of HPV persistence than clearance after LEEP.

Table 3. Results of CD8+ and CD138+ cells expression in patients of CIN with HPV positive before and negative after LEEP (data were presented as number of persistent HR-HPV infection/total in each group)

	Negative (%)	Positive (%)
CD8+ cell		
High: ≥113	18/21 (85.7%)	3/21 (14.29%)
Low: <113	6/13 (46.2%)	7/13 (53.85%)
CD138+ cell		
High: ≥37	5/11 (45.5%)	6/11 (54.55%)
Low: <37	19/22 (86.4%)	3/22 (13.64%)

labeled polymers from the Real Envision Detection kit (Gene Tech #GK500705, Gene Tech Company Limited, Shanghai) for 30 min and visualized by staining with DAB+ chromogen for 20 min. The immunohistochemical results were analyzed using semi-quantitative scoring. The degree and extent of immunopositivity was assessed under a microscope (numerical aperture=0.65, field of vision for the ×40 objective=0.52 mm). The steps were performed according to Ovestad IT [20]. All results were read by 2 experienced pathology experts under supervision of a senior pathologist.

Statistical analysis

Statistical analyses were performed using SPSS (version 13.0, IBM, USA). Fisher's exact test, χ^2, and Logistic regression analysis were used where appropriate. All *P*-values presented are 2-sided and a significant difference was assumed if $P<0.05$.

Results

Patient characteristics

In total, 860 patients who underwent loop electric conization were included in this study. The median age was 33.6 years and ranged from 21 to 65 years. Patient demographics are listed in **Table 1**.

As shown in **Table 1**, the cytology before conization included 231 normal findings (26.9%), 268 ASCUS (31.2%), 80 ASC-H (9.3%), 185 LSILs (21.5%), and 96 HSILs (11.2%). The colposcopy-directed biopsy before conization showed 445 LSIL (51.7%) and 415 HSIL (48.3%). HR-HPV was identified in 759 patients (88.3%) before LEEP. The baseline viral load included 101 (11.7%) patients with negative results, 323 (42.6%) with low loads (RLU/PC=1 to 100), 182 (21.3%) with intermediate loads (100<RLU/PC≤500) and 254 (29.4%) with high loads (500<RLU/PC). The histology of the LEEP conization specimen showed 402 (46.7%) patients with LSIL, 421 (49.0%) with HSIL, 9 (1.0%) with cervical carcinama, and 28 (3.3%) with chronic cervicitis. After LEEP conization, 542 (63%) patients had no detectable high-risk HPV, while 318 (37%) patients had persistent HR-HPV according to the HCII.

Factors associated with HPV persistency after LEEP

Table 2 shows that the HPV clearance rate in different age groups. Rank correlation analysis showed that the virus clearance rate decreased with the increase of age (Rs=-0.109, $P=0.011$). The higher degree of lesion, the higher rate of virus clearance. Positive margin in the LEEP were not associated with HPV clearance/persistency after LEEP conization (**Table 2**). Glands involved was the significant factor for HPV infection clearance after LEEP conization ($X^2=12.145$, $P=0.000$). In a logistic regression analysis, preoperative HPV titer and CIN level were significant factors for persistent HPV infection after LEEP conization (RLU/PC≥500) ($P=0.002$). The HPV infection clearance rate is higher after LEEP in patients with low HPV titer and the high disease level before LEEP (**Table 2**).

Local immune responses and the clearance of HR-HPV after LEEP

The level of IFN-γ expression was associated with the clearance of HR-HPV after LEEP. The HR-HPV clearance rate is low in patients with low level IFN-γ expression (**Figure 1**). CD8+ and

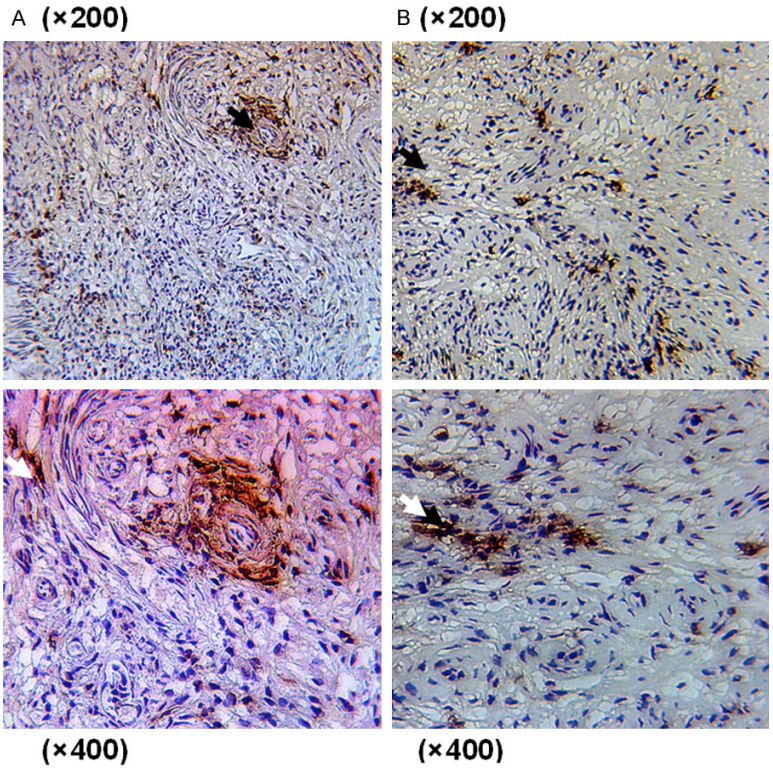

Figure 2. Differences in CD8+ cell expression (A: Few CD8+ cells clearance of HR-HPV infection at 6 months after LEEP; and B: More CD8+ cells persistent HR-HPV infection at 6 months after LEEP).

CD138+ cell numbers are different in cervical tissues without and with persistent HR-HPV infection after LEEP (**Table 3**). The cervical tissues with persistent HR-HPV had fewer CD8+ cells (P=0.022) and more CD138+ cells (P= 0.035) compared with cervical tissues from patients with clearance of HR-HPV after LEEP (**Figures 2**, **3** and **Table 3**).

Discussion

The presence of residual/recurrent disease after LEEP is an important part of postoperative follow-up as nearly all residual/recurrent CIN occurred in patients with HR-HPV persistence [1]. Our study showed that persistent HR-HPV infection associated with age, the severity of the cervical lesions, gland involvement, preoperative HPV titer and local immunity. Condom contraception is an effective method to reduce persistent infection after LEEP. Our results also showed that there are 9 in 860 patients were found with CA after LEEP procedure (1.0%). In 17 patients with CIS after LEEP, 16 of them are HSIL with HPV 16 positive and one of them is HPV 58. In 7 SCC patients, 6 of them are HSIL and 1 is LSIL in the biopsy diagnose before operation. In 1 patient of base cell adenocarcinoma with HPV39 positive the biopsy pathology is not clear but the TCT result is normal. One patient of AIS without HPV type result the biopsy pathology is HSIL before operation.

Several studies reported factors associated with the clearance of HPV infection after CIN treatment with, however, in consistent conclusions. Some studies have shown that age is not related to the persistence of HPV after treatment [18, 19], which is contrary to this study. Studies of the persistency of HPV infection after successful LEEP had inconsistent results [27-33]. Whether age is related to the clearance of HPV following LEEP remains controversial [28-30]. Park et al. reported that CIN grade was not a predictive factor for HPV persistency [31]. Our result showed that a low grade of CIN was a factor in predicting HPV persistency. This result may imply that LEEP operation is not the best way for LSIL.

To authors' best knowledge, the cervical local immune response index as a risk factor for predicting the persistence of HPV infection has not been reported previously. In our study, persistent HR-HPV infections after LEEP were most likely to occur when there were lower numbers of CD8+ cells, higher numbers of CD138+ cells and lower levels of IFN-γ in the cervical microenvironment. Therefore, these factors have predictive value in clinical treatment. Differences in IFN-γ mRNA levels in patients with HPV clearance and with HPV persistence after successful LEEP have not been previously reported. Scott et al. reported that a Th1 cytokine response occurred in the cervical tissue with the clearance of HPV infection [34]. El-Sherif et al. demonstrated that IFN-γ mRNA ex-

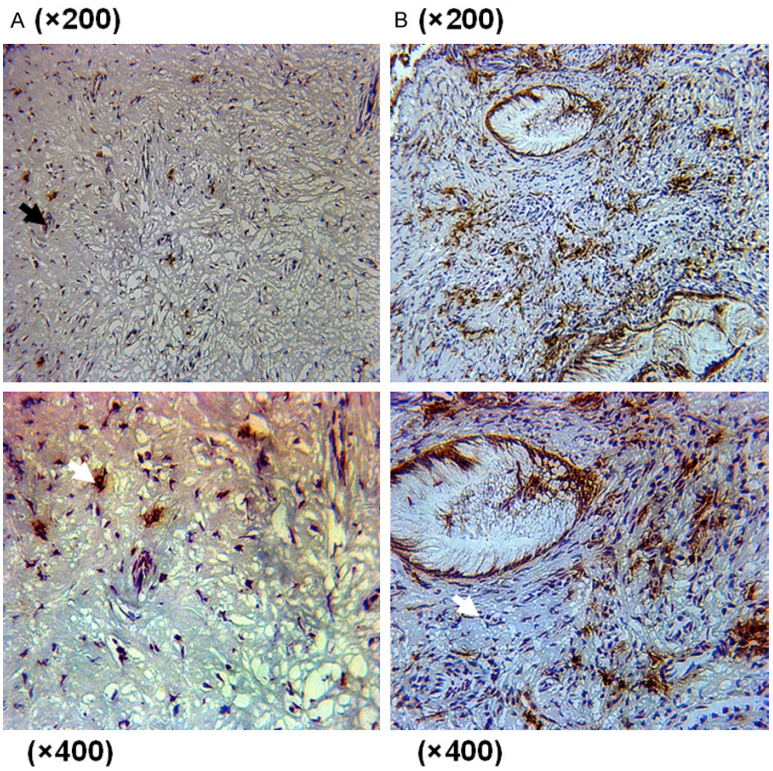

Figure 3. Differences in CD138+ cell expression (A: Few CD138+ cells expression clearance of HR-HPV infection at 6 months after LEEP; and B: More CD138+ cells expression persistent HR-HPV infection at 6 months after LEEP).

pression was significantly higher in normal cervical tissue than in cervical tissue with CIN [35]. Studies of the cervical local immune response have shown that adaptive immune cells infiltrate CIN lesions. CD8+ cytotoxic T-lymphocytes and CD138+ B-lymphocytes are adaptive immune cells. The E6 and E7 proteins of HPV-infected cells are recognized and act specifically against these adaptive immune cells [20]. Ovestal et al. demonstrated that high-grade CIN was likely to regress due to the specific reaction of adaptive immune cells. Higher CD8+ cytotoxic T-lymphocyte numbers and lower CD138+ B-lymphocyte numbers are present in cases of lesion regression [36].

In conclusion, HR-HPV infection cleared after successful LEEP conization in most patients. HPV DNA loads >500 RLU/PC, age, without condom controception and low local immunity are important high risk factors of HPV persistence after LEEP. Local immune status in patients with HPV persistent infection needs further studies.

Acknowledgements

This work was supported by Shanghai Science and Technology Committee Foundation (Grant NO. 1541196-7700), and National Natural Science Foundation of China (Grant NO. 81572546).

Disclosure of conflict of interest

None.

Address correspondence to: Jing Sun, Department of Gynecology, Cervical Disease Centre, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China. E-mail: sunjing61867@126.com; Fang Li, Department of Gynecology, Cervical Disease Centre, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China. Tel: +86-21 5403 5206; E-mail: fang_li@tongji.edu.cn

References

[1] Kim YT, Lee JM, Hur SY, Cho CH, Kim YT, Kim SC and Kang SB. Clearance of human papillomavirus infection after successful conization in patients with cervical intraepithelial neoplasia. Int J Cancer 2010; 126: 1903-1909.

[2] Aerssens A, Claeys P, Garcia A, Sturtewagen Y, Velasquez R, Vanden Broeck D, Vansteelandt S, Temmerman M and Cuvelier CA. Natural history and clearance of HPV after treatment of precancerous cervical lesions. Histopathology 2008; 52: 381-386.

[3] Giorgi-Rossi P, Franceschi S and Ronco G. HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. Int J Cancer 2012; 130: 1387-1394.

[4] Nam K, Chung S, Kim J, Jeon S and Bae D. Factors associated with HPV persistence after conization in patients with negative margins. J Gynecol Oncol 2009; 20: 91-95.

[5] Chen HC, Schiffman M, Lin CY, Pan MH, You SL, Chuang LC, Hsieh CY, Liaw KL, Hsing AW, Chen CJ; CBCSP-HPV Study Group. Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. J Natl Cancer Inst 2011; 103: 1387-1396.

[6] zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009; 384: 260-265.
[7] Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D; 2006 American Society for Colposcopy and Cervical Pathology-sponsored Consensus Conference. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. J Low Genit Tract Dis 2007; 11: 223-239.
[8] Kang WD, Oh MJ, Kim SM, Nam JH, Park CS and Choi HS. Significance of human papillomavirus genotyping with high-grade cervical intraepithelial neoplasia treated by a loop electrosurgical excision procedure. Am J Obstet Gynecol 2010; 203: 72, e71-76.
[9] Alonso I, Torne A, Puig-Tintore LM, Esteve R, Quinto L, Campo E, Pahisa J and Ordi J. Pre- and post-conization high-risk HPV testing predicts residual/recurrent disease in patients treated for CIN 2-3. Gynecol Oncol 2006; 103: 631-636.
[10] Costa S, Simone PD, Venturoli S, Cricca M, Zerbini ML, Musiani M, Terzano P, Santini D, Cristiani P, Syrjänen S and Syrjänen K. Factors predicting human papillomavirus clearance in cervical intraepithelial neoplasia lesions treated by conization. Gynecol Oncol 2003; 90: 358-365.
[11] Nagai Y, Maehama T, Asato T and Kanazawa K. Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence? Gynecol Oncol 2000; 79: 294-299.
[12] Acladious NN, Sutton C, Mandal D, Hopkins R, Zaklama M and Kitchener H. Persistent human papillomavirus infection and smoking increase risk of failure of treatment of cervical intraepithelial neoplasia (CIN). Int J Cancer 2002; 98: 435-439.
[13] Malapati R, Chaparala S, Cejtin HE. Factors influencing persistence or recurrence of cervical intraepithelial neoplasia after loop electrosurgical excision procedure. J Low Genit Tract Dis 2011; 15: 177-179.
[14] Leguevaque P, Motton S, Decharme A, Soulé-Tholy M, Escourrou G, Hoff J. Predictors of recurrence in high-grade cervical lesions and a plan of management. Eur J Surg Oncol 2010; 36: 1073-1079.
[15] Valasoulis G, Koliopoulos G, Founta C, Kyrgiou M, Tsoumpou I, Valari O, Martin-Hirsch P, Daponte A, Karakitsos P, Paraskevaidis E. Alterations in human papillomavirus-related biomarkers after treatment of cervical intraepithelial neoplasia. Gynecol Oncol 2011; 121: 43-48.
[16] Prato B, Ghelardi A, Gadducci A, Marchetti I, Di Cristofano C, Di Coscio G, Bevilacqua G, Genazzani AR. Correlation of recurrence rates and times with posttreatment human papillomavirus status in patients treated with loop electrosurgical excision procedure conization for cervical squamous intraepithelial lesions. Int J Gynecol Cancer 2008; 18: 90-94.
[17] Visser J, van Baarle D, Hoogeboom BN, Reesink N, Klip H, Schuuring E, Nijhuis E, Pawlita M, Bungener L, de Vries-Idema J, Nijman H, Miedema F, Daemen T, van der Zee A. Enhancement of human papilloma virus type 16 E7 specific T cell responses by local invasive procedures in patients with (pre)malignant cervical neoplasia. Int J Cancer 2006; 118: 2529-2537.
[18] Piersma SJ, Jordanova ES, van Poelgeest MI, Kwappenberg KM, van der Hulst JM, Drijfhout JW, Melief CJ, Kenter GG, Fleuren GJ, Offringa R, van der Burg SH. High number of intraepithelial CD8+ tumor-infiltrating lymphocytes is associated with the absence of lymph node metastases in patients with large early-stage cervical cancer. Cancer Res 2007; 67: 354-361.
[19] Mace TA, Zhong L, Kokolus KM and Repasky EA. Effector CD8+ T cell IFN-gamma production and cytotoxicity are enhanced by mild hyperthermia. Int J Hyperthermia 2012; 28: 9-18.
[20] Ovestad IT, Gudlaugsson E, Skaland I, Malpica A, Kruse AJ, Janssen EA and Baak JP. Local immune response in the microenvironment of CIN2-3 with and without spontaneous regression. Mod Pathol 2010; 23: 1231-1240.
[21] Clerici M, Merola M, Ferrario E, Trabattoni D, Villa ML, Stefanon B, Venzon DJ, Shearer GM, De Palo G, Clerici E. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. J Natl Cancer Inst 1997; 89: 245-250.
[22] Stanley M. Immunobiology of HPV and HPV vaccines. Gynecol Oncol 2008; 109: S15-21.
[23] Igawa T, Sato Y, Kawai H, Kondo E, Takeuchi M, Miyata-Takata T, Takata K and Yoshino T. Spontaneous regression of plasmablastic lymphoma in an elderly human immunodeficiency virus (HIV)-negative patient. Diagn Pathol 2015; 10: 183.
[24] Scott ME, Ma Y, Kuzmich L and Moscicki AB. Diminished IFN-gamma and IL-10 and elevated Foxp3 mRNA expression in the cervix are associated with CIN 2 or 3. Int J Cancer 2009; 124: 1379-1383.
[25] Khatissian E,Monceaux V, Cumont MC, Montagnier L, Hurtrel B, Chakrabarti L. Interferon-gamma expression in macaque lymph nodes during primary infection with simian immunodeficiency virus. Cytokine 1996; 8: 844-852.
[26] Lee HS, Choe G, Park KU, Park DJ, Yang HK, Lee BL, Kim WH. Altered expression of DNA-

dependent protein kinase catalytic subunit (DNA-PKcs) during gastric carcinogenesis and its clinical implications on gastric cancer. Int J Oncol 2007; 31: 859-866.

[27] Castle PE, Kreimer AR, Wacholder S, Wheeler CM, Koutsky LA, Rydzak G, Buckman DW, Graubard B and Schiffman M. Influence of loop electrosurgical excision procedure on subsequent acquisition of new human papillomavirus infections. J Infect Dis 2009; 199: 1612-1620.

[28] Sarian LO, Derchain SF, Pitta Dda R, Morais SS and Rabelo-Santos SH. Factors associated with HPV persistence after treatment for high-grade cervical intra-epithelial neoplasia with large loop excision of the transformation zone (LLETZ). J Clin Virol 2004; 31: 270-274.

[29] Rositch AF, Silver MI, Burke A, Viscidi R, Chang K, Duke CM, Shen W and Gravitt PE. The correlation between human papillomavirus positivity and abnormal cervical cytology result differs by age among perimenopausal women. J Low Genit Tract Dis 2013; 17: 38-47.

[30] Dalstein V, Riethmuller D, Pretet JL, Le Bail Carval K, Sautiere JL, Carbillet JP, Kantelip B, Schaal JP and Mougin C. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer 2003; 106: 396-403.

[31] Park JY, Lee KH, Dong SM, Kang S, Park SY and Seo SS. The association of pre-conization high-risk HPV load and the persistence of HPV infection and persistence/recurrence of cervical intraepithelial neoplasia after conization. Gynecol Oncol 2008; 108: 549-554.

[32] Serati M, Siesto G, Carollo S, Formenti G, Riva C, Cromi A and Ghezzi F. Risk factors for cervical intraepithelial neoplasia recurrence after conization: a 10-year study. Eur J Obstet Gynecol Reprod Biol 2012; 165: 86-90.

[33] Kim JW, Song SH, Jin CH, Lee JK, Lee NW, Lee KW. Factors affecting the clearance of high-risk human papillomavirus infection and the progression of cervical intraepithelial neoplasia. J Int Med Res 2012; 40: 486-496.

[34] Scott M, Stites DP, Moscicki AB. Th1 cytokine patterns in cervical human papillomavirus infection. Clin Diagn Lab Immunol 1999; 6: 751-755.

[35] El-Sherif AM, Seth R, Tighe PJ and Jenkins D. Quantitative analysis of IL-10 and IFN-gamma mRNA levels in normal cervix and human papillomavirus type 16 associated cervical precancer. J Pathol 2001; 195: 179-185.

[36] Ovestad IT, Gudlaugsson E, Skaland I, Malpica A, Munk AC, Janssen EA and Baak JP. The impact of epithelial biomarkers, local immune response and human papillomavirus genotype in the regression of cervical intraepithelial neoplasia grades 2-3. J Clin Pathol 2011; 64: 303-307.