Gineco.eu [11] 176-179 [2015]
DOI: 10.18643/gieu.2015.176

Risk factors for high-risk human papilloma virus persistence after loop excision procedure as treatment of cervical dysplasia

Ramona Gabriela Ursu[1], Ana Cristina Anton[2], Dragos Nemescu[2], Luminita Smaranda Iancu[1]

1. Department of Microbiology, "Gr.T.Popa" University of Medicine and Pharmacy Iasi, Romania
2. Department of Obstetrics & Gynecology, "Gr.T.Popa" University of Medicine and Pharmacy Iasi, Romania

Correspondence:
Dr. Dragos Nemescu
e-mail: dragos.nemescu@umfiasi.ro

Acknowledgement:
This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893.

Abstract

Persistence of high-risk human papilloma virus types (HR-HPV) after surgical treatment of cervical intraepithelial neoplasia (CIN) is an important factor which influences the management and the recurrence risk. In this study, we assessed the efficiency of the loop electrosurgical excision procedure (LEEP) in HR-HPV removal. Cervical samples from 31 women, diagnosed with CIN and HR-HPV, were genotyped at six months after LEEP. We assessed the influence of various risk factors on HR-HPV persistence, using univariate and multivariate analysis: age, menopausal status, parity, abortions, oral contraception, smoking, sexual partners, initial cervical smear test and histopathological results. We detected persistent infections in 7 (22.6%) patients with 16, 18, 31, 39, 51 and 66 HPV types. Univariate analysis found that age over 30 years, multiparity, use of contraception and CIN2-3 were significant factors for persistence of HR-HPV after LEEP. Multivariate analysis showed that CIN2-3 was the only significant risk factor for HPV persistence (OR=10.7). Furthermore, although not significant, parity was also retained into final equation. HR-HPV persistence is a frequent phenomenon after LEEP. We highlight the importance of glandular involvement, residual tissue and difficulties of the resection procedure in multipara. HPV genotyping is a sensitive method to follow up this group of patients, as it can identify a type specific HPV infection. In our country with the known highest mortality rate in EU countries of cervical cancer, we need an organized cervical neoplasia screening with a validated HPV genotyping test.
Keywords: *human papillomavirus, genotyping, cervical intraepithelial neoplasia, persistence, cervical cancer*

Introduction

Infection with persistent high-risk risk human papilloma virus (HR-HPV) type can lead to cervical cancer[1]. Like a preventive measure, in case of colposcopy confirmed diseases, one can apply some strategies like destructive or excisional procedures. The lasts are preferred in the majority of cases because they are obvious superior in comparison with the destructive methods, having the advantage of histological evaluation of the transformation area. The histological examination of the excised tissue allows the pathologist to assess the presence of a microinvasive cancer[2].

The purpose of the excisional therapy is to remove the entire lesion. The excision of transformation area is not indicated for cervical intraepithelial neoplasia (CIN)1, only if this lesion had persisted more than one year. This surgical procedure should be applied as soon as possible if there is CIN2–3 or a risk for invasion. The techniques used for a complete excision are: large loop excision of the transformation zone/loop electrosurgical excision procedure (LLEP), cold knife conization, laser excision and needle excision of the transformation zone[3,4]. Usually, the therapy for CIN2 is efficient, but there is still a risk for evolution to invasive cancer in 20 years after therapy[5].

Recommended follow-up procedures after CIN therapy are Pap smear and HPV (deoxyribonucleic acid) DNA typing[3]. However, the efficiency of the cytology in follow-up raised many controversies[6]. As it is known that HPV infection is essential for developing and maintaining of CIN, the DNA/HPV detection can highlight the residual/recurrent CIN lesions faster and with a higher sensitivity[7]. Some studies have concluded that the persistence of DNA/HPV is predictive for recurrence of the disease[6-8]. Moreover, testing for DNA/HPV can be used to assess different methods of therapy, with variable rate of clearance of viral genome[9].

In Arbyn's et al. meta-analyses, that assessed the clinical utility of DNA/HPV testing, the histology exam had a failure prediction after CIN lesion therapy of 56.6%. Using only cytology, the prediction values raised to 75.9%. DNA/HPV testing had a recurrence prediction of 95.9%[10].

Thus, HR-HPV detection after therapy predicts recurrent CIN with a higher sensitivity in comparison with Pap smear or with histological examination. The specificity of HR-HPV

Received:
October 22, 2015
Revised:
November 06, 2015
Accepted:
November 28, 2015

testing is not different by histology, but it is lower than the repeated Pap smear. HPV testing, by its high sensitivity, can be the best modality to avoid the recurrent disease in a long term follow-up[11-13].

The aims of our study were to assess the efficiency of excisional procedure in removal of HR-HPV load among CIN positive patients and to investigate predictive risk factors for HR-HPV persistence after the LEEP.

Methods

This was a retrospective study which included 31 women referred to our laboratory, for HPV genotyping, between January 2012 and December 2014, at six months after LEEP. All patients with positive for HR-HPV at pre-treatment visit were eligible for the study. Exclusion criteria were a later diagnosis of invasive cervical cancer, next total hysterectomy, pregnancy, clinical signs of immunosuppression, human immunodeficiency virus positive and unavailability of required data.

We recorded in each case patient age, menopausal status, parity, number of abortions, oral contraceptive use, smoking condition, number of sexual partners, initial Pap test and histopathological results.

Consultation routine. All patients were recruited by an opportunistic screening for the prevention of cervical neoplasia. At the pre-treatment visit, all patients filled a questionnaire regarding social and demographic data, and a complete gynecological examination was performed. These were followed by colposcopy and collection of endocervical specimens for HPV genotyping. If it was identified an abnormality suggestive for CIN2 or worse on the cervix, a biopsy LEEP was performed.

At 6 months after LEEP, cervical cytology for Pap test and HPV genotyping was collected using a cervix brush and processed using a liquid-based approach. An experienced pathologist, blinded to the results of the HPV test, performed histopathological and cytological examination.

LEEP. The cervix and transformation zone were evaluated by colposcopy. Assessment of the transformation zone was enhanced using Lugol's iodine solution. The electrosurgical excision of the whole transformation zone was performed with a wire loop of appropriate size, by an experienced gynecologist specialized in colposcopy, as described previously[14,15]. Local anesthesia was implemented with 2% lidocaine inoculated with epinephrine in every quadrant. Excision depth was dependent on clinical judgement, in accordance with the characteristics of the lesion (i.e. size, completely visible by colposcopy) and cervix (i.e. length, opening).

HPV genotyping. The gynecologist collected cervical cells in Cobas PCR Cell Collection Media (Roche, Romania) from all the women, and then kept at 4°C till processing (1-7 days). For DNA/HPV purification, we used High Pure PCR template (Roche, Romania) and for HPV genotyping, we used Linear Array Genotyping Test (Roche, Romania), previously described. Linear Array supposed one step of PCR amplification and one of the hybridizations of amplicons. The genotyping test is detecting single or multiple infections of 37 HPV genotypes: 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and 24 low risk and intermediary risk types (6, 11, 26, 39, 40, 42, 45, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108). The presence of β-globin (low and high intensity) on each strip is a control for a correct sampling, DNA/HPV purification and amplification[16].

Statistical Analysis. Data were analyzed with the SPSS version 21.0 program (IBM Corporation, Armonk, NY). Univariate analyses to identify variables associated with HPV persistence were performed using $\chi 2$, Fisher exact, Student's t, and Mann-Whitney U tests, as appropriate. Two-sided $p< 0.05$ was considered statistically significant. For multivariate analysis, possible factors identified in the univariate analyses were further entered into the logistic regression model to determine independent predictors of HPV persistence. We applied backward stepwise binary logistic regression, using Wald $p<0.05$ for entry and $p>0.1$ for removal.

Results

A total of 31 women with significant cervical lesions and treated with LEEP were eligible for the study. Median patient age was 32 years (interquartile range, 10). Nine (29%) women were nulliparous and 19 (71%) had at least 1 prior delivery. Fourteen patients (45.2%) were chronic tobacco consumers, 10 (32.3%) declared having 3 or more sexual partners and 15 (48.4%) used oral contraception in the last 5 years. Only one woman was postmenopausal.

Initial cytological examination revealed 6 (19.4%) normal Pap test, 5 (16.1%) ***atypical squamous cells*** of undetermined significance, 3 (9.7%) atypical squamous cells of undetermined significance, a high-grade squamous intraepithelial lesion is not excluded as a possibility, 11 (35.5%) low grade squamous intraepithelial lesions and 6 (19.4%) high-grade squamous intraepithelial lesions. Before surgery, all patients were positive for high-risk HPV/DNA. The most frequent single HPV type infections before surgery were with HPV 16 and 18 (each 4 / 12.9%), followed by single infections with the HPV types 31, 51, 53, 83, and by multiple infections with HPV 31, 51, 54; 31, 66; 51, 61; 52, 58, 62 and 6, 39.

Histological evaluation of the excision specimens showed 17 (54.8%) women with CIN1, 13 (41.9%) with CIN2 and one (3.2%) with CIN3. Post LEEP, at six months, persistence of HR- HPV/DNA was detected in 7 (22.6%) women: one case each with HPV 16, 18, 31, 39, 51, 66 respectively.

We further compared the frequency of risk factors in women with and without persistent HPV infection (Table 1). Univariate analysis found that age over 30 years, parity, use of contraception and CIN2-3 were significant factors for persistence of high-risk HPV after LEEP. These factors were included in the logistic regression analysis model. Multivariate analysis shown that CIN2-3 was the only significant risk factor for HPV persistence (OR=10.7, 95% CI 1.22-116.23). The classification of other risk factors, in order of decreasing importance, was: increasing parity, use of oral contraception and age >30 years, respectively (Table 2).

Discussion

CIN is a precursor of cervical cancer and women with high-grade lesions like CIN 2-3 have a variable risk for developing invasive disease[17]. An effective therapeutic option to treat

the viral infection is not yet available and therefore, excisional methods are usually implemented: LEEP, cold knife conization and laser ablation. These surgical methods aim to eliminate the HPV infection causing the cervical abnormality and have shown an effective therapy for high-grade CIN. All three methods available have a comparable efficiency[15,18-20]. LEEP has some advantages: performed under local anesthesia, in an office setting, provide most reliable specimens for histology and preserve fertility[21,22].

Women treated for CIN have a significant risk of recurrence and invasive disease, five times higher than for the general population[5]. Therefore, patients who underwent surgical excision must be followed closely.

Recent studies have shown that the persistence of HR-HPV is the main cause for recurrent/residual disease in this group. HPV testing is useful as an adjunct to cervical cytology in detecting patients with increasing risk of persistence or recurrence of CIN2-3 following conization[11,15,23-25]. Accordingly actual guidelines the follow-up of women with biopsy - confirmed high-grade CIN should start at 6 month after surgical intervention, by Pap smear and HPV genotyping[26]. In HPV-negative women after conization, the risk for high-grade CIN in first 5 years is similar to that of HPV-negative women in the general population[27].

We assessed the efficiency of the LEEP in case of HR-HPV and CIN positive patients, by HPV/DNA testing at 6 months. In our series, the HPV positivity has reduced from 100% before LEEP to 22.6% at 6 months following the procedure. The HPV persistence was significantly higher in CIN2-3 patients (43%) compared to CIN1 group (5.9%) (Table 1).

Table 1 Associations between post-conization HPV persistence and clinico-pathologic study parameters

Variable	HPV negative (n=27)	HR HPV (n=7)	p	OR (95% CI)
Age>30 years	13 (54.2%)	7 (100%)	p=0.033	1.54 (1.12-2.12)
Parity	0.5 (0-2)	1 (1-3)	p=0.036 *	-
Abortions	1 (0-3)	2 (0-2)	NS *	-
Sex partners ≥3	7 (29.2%)	3 (42.9%)	NS	1.82 (0.32-10.34)
Contraception	9 (37.5%)	6 (85.7%)	p=0.04	10 (1.03-97.04)
Menopause	0 (0%)	1 (14.3%)	NS	-
Smoking	10 (41.7%)	4 (57.1%)	NS	1.87 (0.34-10.25)
CIN2-CIN3	8 (33.3%)	6 (85.7%)	p=0.03	12 (1.22- 117.4)

Data are shown as number (%) or median. CIN= cervical intraepithelial neoplasia; CI= confidence interval; OR= odds ratio; NS= not significant.
** Mann Whitney U test. Rest of comparisons performed with Fisher's exact or χ2 tests.*

Table 2 Results of logistic regression analysis

Variable	Odds ratio (95% confidence interval)	p
Age>30 years	NE*	NE*
CIN2-CIN3	10.7 (1.22-116.23)	p=0.05
Contraception	NE*	NE*
Parity	2.96 (0.83-10.49)	0.094

**NE= not in the equation*

The reported rate of HR-HPV positivity following excisional methods varies enormously[24]. A literature search found the persistence of high-risk HPV in CIN2-3 patients, at 6 months after LEEP/LLETZ, between 25% and 51.4%(15,28-31). Our incidence is at the upper limit of this interval and supports that the risk of residual/recurrent diseases is not negligible. Even in patients with negative margins of resection, the HR-HPV persistence rate varies between 17.8% and 28.8% at 6 and 24 months, respectively[32,33].

Therefore, identification of risk factors that could predict which women are at risk for HPV persistence after treatment were of great clinical importance. In our series, univariate analysis found that age over 30 years, multiparity, use of contraception and CIN2-3 were significant factors for persistence of HR HPV after LEEP. Our multivariate analysis shown that CIN2-3 was the only relevant risk factor for HPV persistence (OR=10.7). Additionally, although not significant, parity was also retained into final equation.

In other studies, HR-HPV persistence in CIN2+ treated patients was associated with smoking and age over 35 years, irrespective of margins' status[34], infection with HPV α3 species and an extensive initial disease[35]. The pre-intervention high HPV load was found as a risk factor for HR-HPV persistence[36], also in patients with negative excision margins[33].

In a recent study, Baser and cntributors found that patient age over 30 years, multiparity (>1) and cone depth (>=15 mm) were significant factors for HR-HPV persistence at 12 months, in CIN2+ patients. Their multivariate analysis showed also that patient age and cone depth were significant predictors of HPV/DNA persistence[37].

These relatively common risks factors highlight the importance of glandular involvement, residual tissue and difficulties of the resection procedure in multipara.

Conclusions

Linear Array HPV Genotyping is a suitable assay to follow up surgical treated women for CIN, as it can identify type specific infections. Our study contributes to knowledge of the natural history of HPV infection after conization. Risk factors associated with persistence of HR-HPV after LEEP are a high grade of cervical displasia (CIN2+), multiparity, use of oral contraception and age >30 years. We recommend the HPV testing to be routinely performed after LEEP. ■

References

1. Wright TC Jr, Massad LS, Dunton CJ. et al. 2006 consensus guidelines for the management of women with abnormal cervical screening tests. Journal of lower genital tract disease 2007. 11, 201-22.
2. Arbyn M, Anttila A, Jordan J. et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition--summary document. Annals of oncology. Official journal of the European Society for Medical Oncology / ESMO 2010, 21, 448-58.
3. Jordan J, Martin-Hirsch P, Arbyn M. et al. European guidelines for clinical management of abnormal cervical cytology, part 2. Cytopathology: Official Journal of the British Society for Clinical Cytology 2009, 20, 5-16.
4. Jordan J, Arbyn M, Martin-Hirsch P. et al. European guidelines for quality assurance in cervical cancer screening: recommendations for clinical management of abnormal cervical cytology, part 1. Cytopathology. Official Journal of the British Society for Clinical Cytology 2008, 19, 342-54.
5. Soutter WP, de Barros Lopes A, Fletcher A. et al. Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia. Lancet 1997, 349, 978-80.
6. Kocken M, Uijterwaal MH, de Vries AL. et al. High-risk human papillomavirus testing versus cytology in predicting post-treatment disease in women treated for high-grade cervical disease: a systematic review and meta-analysis. Gynecologic oncology 2012, 125, 500-7.
7. Gosvig CF, Huusom LD, Deltour I. et al. Role of human papillomavirus testing and cytology in follow-up after conization. Acta obstetricia et gynecologica Scandinavica 2015, 94, 405-11.
8. Enache LSE, Borda, A, Dobreanu M. Human papillomaviruses and cervical cancer. Rev Romana Med Lab 2007. 8, 19.
9. Strander B, Ryd W, Wallin KL. et al. Does HPV-status 6-12 months after treatment of high grade dysplasia in the uterine cervix predict long term recurrence? European Journal of Cancer 2007, 43, 1849-55.
10. Arbyn M, Paraskevaidis E, Martin-Hirsch P. et al. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. Gynecologic oncology 2005, 99, S7-11.
11. Vintermyr OK, Iversen O, Thoresen S. et al. Recurrent high-grade cervical lesion after primary conization is associated with persistent human papillomavirus infection in Norway. Gynecologic oncology 2014, 133, 159-66.
12. Soderlund-Strand A, Kjellberg L, Dillner J. Human papillomavirus type-specific persistence and recurrence after treatment for cervical dysplasia. Journal of medical virology 2014, 86, 634-41.
13. Distefano AL, Picconi MA, Alonio LV. et al. Persistence of human papillomavirus DNA in cervical lesions after treatment with diathermic large loop excision. Infectious diseases in obstetrics and gynecology 1998, 6, 214-9.
14. Sarian LO, Derchain SF, Andrade LA. et al. HPV DNA test and Pap smear in detection of residual and recurrent disease following loop electrosurgical excision procedure of high-grade cervical intraepithelial neoplasia. Gynecologic oncology 2004, 94, 181-6.
15. van Ham MA, van Hamont D, Bekkers RL. et al. High-risk HPV presence in cervical specimens after a large loop excision of the cervical transformation zone: significance of newly detected hr-HPV genotypes. Journal of medical virology 2007, 79, 314-9.
16. Ursu RG, Onofriescu M, Nemescu D. et al. HPV prevalence and type distribution in women with or without cervical lesions in the Northeast region of Romania. Virology journal 2011, 8, 558.
17. Moscicki AB, Schiffman M, Burchell A. et al. Updating the natural history of human papillomavirus and anogenital cancers. Vaccine 2012, 30 Suppl 5, F24-33.
18. Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. International journal of cancer Journal international du cancer 2006, 118, 2048-55.
19. Orbo A, Arnesen T, Arnes M. et al. Resection margins in conization as prognostic marker for relapse in high-grade dysplasia of the uterine cervix in northern Norway: a retrospective long-term follow-up material. Gynecologic oncology 2004, 93, 479-83.
20. Johnson N, Khalili M, Hirschowitz L. et al. Predicting residual disease after excision of cervical dysplasia. BJOG: an international journal of obstetrics and gynaecology 2003, 110, 952-5.
21. Martin-Hirsch PP, Paraskevaidis E, Bryant A. et al. Surgery for cervical intraepithelial neoplasia. The Cochrane database of systematic reviews 2013, 12, CD001318.
22. Jancar N, Rakar S, Poljak M. et al. Efficiency of three surgical procedures in eliminating high-risk human papillomavirus infection in women with precancerous cervical lesions. European journal of gynaecological oncology 2006, 27, 239-42.
23. Mo LZ, Song HL, Wang JL. et al. Pap Smear Combined with HPV Testing: A Reasonable Tool for Women with High-grade Cervical Intraepithelial Neoplasia Treated by LEEP. Asian Pacific journal of cancer prevention : APJCP 2015, 16, 4297-302.
24. Rositch AF, Soeters HM, Offutt-Powell TN. et al. The incidence of human papillomavirus infection following treatment for cervical neoplasia: a systematic review. Gynecologic oncology 2014, 132, 767-79.
25. Park JY, Bae J, Lim MC. et al. Role of high risk-human papilloma virus test in the follow-up of patients who underwent conization of the cervix for cervical intraepithelial neoplasia. Journal of gynecologic oncology 2009;20:86-90.
26. Massad LS, Einstein MH, Huh WK. et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstetrics and gynecology 2013, 121, 829-46.
27. Gosvig CF, Huusom LD, Andersen KK. et al. Long-term follow-up of the risk for cervical intraepithelial neoplasia grade 2 or worse in HPV-negative women after conization. International journal of cancer Journal international du cancer 2015.
28. Leguevaque P, Motton S, Decharme A. et al. Predictors of recurrence in high-grade cervical lesions and a plan of management. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology 2010, 36, 1073-9.
29. Baloglu A, Uysal D, Bezircioglu I. et al. Residual and recurrent disease rates following LEEP treatment in high-grade cervical intraepithelial lesions. Archives of gynecology and obstetrics 2010, 282, 69-73.
30. Venturoli S, Ambretti S, Cricca M. et al. Correlation of high-risk human papillomavirus genotypes persistence and risk of residual or recurrent cervical disease after surgical treatment. Journal of medical virology 2008, 80, 1434-40.
31. Fambrini M, Penna C, Pieralli A. et al. CO2 laser cylindrical excision or standard re-conization for persistent-recurrent high-grade cervical intraepithelial neoplasia (HG-CIN) in women of fertile age. Anticancer research 2008, 28, 3871-5.
32. Wu D, Zheng Y, Chen W. et al. Prediction of residual/recurrent disease by HPV genotype after loop excision procedure for high-grade cervical intraepithelial neoplasia with negative margins. The Australian & New Zealand journal of obstetrics & gynaecology 2011, 51, 114-8.
33. Nam K, Chung S, Kim J. et al. Factors associated with HPV persistence after conization in patients with negative margins. Journal of gynecologic oncology 2009, 20, 91-5.
34. Sarian LO, Derchain SF, Pitta Dda R. et al. Factors associated with HPV persistence after treatment for high-grade cervical intra-epithelial neoplasia with large loop excision of the transformation zone (LLETZ). Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology 2004, 31, 270-4.
35. Kreimer AR, Katki HA, Schiffman M. et al. Viral determinants of human papillomavirus persistence following loop electrical excision procedure treatment for cervical intraepithelial neoplasia grade 2 or 3. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2007, 16, 11-6.
36. Park JY, Lee KH, Dong SM. et al. The association of pre-conization high-risk HPV load and the persistence of HPV infection and persistence/recurrence of cervical intraepithelial neoplasia after conization. Gynecologic oncology 2008, 108, 549-54.
37. Baser E, Ozgu E, Erkilinc S. et al. Risk factors for human papillomavirus persistence among women undergoing cold-knife conization for treatment of high-grade cervical intraepithelial neoplasia. International Journal of Gynecology & Obstetrics 2014, 125, 275-78.