Efficiency of three surgical procedures in eliminating high-risk human papillomavirus infection in women with precancerous cervical lesions

N. Jančar[1], S. Rakar[1], M. Poljak[2], K. Fujs[2], B.J. Kocjan[2], E. Vrtačnik-Bokal[1]

[1]Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana
[2]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana (Slovenia)

Summary

Purpose of investigation: To establish the efficiency of laser vaporization (LV), large loop excision of the transformation zone (LLETZ) and cold knife conization, done for precancerous cervical lesions, in eliminating high-risk human papillomavirus (HPV) infection. Additionally, we determined whether the same HPV genotype persisted after surgery.

Methods: A total of 214 women were tested for HPV infection by the Hybrid Capture II (HCII) test prior to surgery. HPV-positive women were followed by HCII test ten months after surgery. In persistently HPV-positive women, HPV genotypes were determined by PCR – PGMY09/PGMY11.

Results: The HCII test showed elimination of HPV infection after LV, LLETZ and cold knife conization in 67.6%, 86.3%, and 100% ($p < 0.05$) of women, respectively. In seven (38.9%) women a different HPV genotype was found to be present after surgery, the corrected efficiency thus being 79.4%, 92.7% and 100% (p = NS), respectively.

Conclusions: The three analyzed surgical procedures are effective in eliminating high-risk HPV infection. HPV testing is useful at follow-up, since it can identify a small proportion of women requiring close surveillance and potential treatment.

Key words: Human papillomavirus; Precancerous cervical lesions; Surgical treatment; Follow-up.

Introduction

Cervical cancer is regarded as the most preventable and treatable form of cancer. This can be achieved by early diagnosis and treatment of precancerous lesions [1].

For many years, cytological testing has been the standard screening procedure for cervical cancer. Persistent infection with high-risk human papillomavirus (HPV) genotypes is known to be a necessary etiological factor for the development of cervical cancer [2, 3]. Therefore, testing for infection with high-risk HPV genotypes has been introduced as a complementary method to cytology. The Hybrid Capture II (HCII) HPV DNA Test (Digene Corp., Gaithersburg, MD, USA) is widely used for the detection of HPV infection [4-11]. Despite the recommendations for the management of women with abnormal smears [6], testing for HPV by HCII is still not sufficiently used in Slovenia. Some studies [12-15] have confirmed that HPV testing is useful also for follow-up after treatment of precancerous cervical lesions.

The most frequently used surgical procedures for the treatment of precancerous cervical lesions at the Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, are laser vaporization (LV), large loop excision of the transformation zone (LLETZ), and cold knife conization. Although the guidelines for the management of women with cervical intraepithelial neoplasia (CIN) have been developed [7], indications for certain procedures are not always clear, and the decision on the treatment modality to be used in a specific patient lies in the hands of the clinician.

The aim of this study was to establish the efficiency of LV, LLETZ and cold knife conization in eliminating infection with high-risk HPV genotypes; the presence or absence of HPV infection was detected by the HCII test. Furthermore, we wanted to find whether the same HPV genotype persisted after surgery using restriction fragment length polymorphism analysis of polymerase chain reaction (PCR) products with PGMY09/PGMY11 primers.

Methods

This clinical prospective study was carried out between November 2002 and May 2004 at the Department of Obstetrics and Gynecology, University Medical Centre Ljubljana. We included 214 consecutive women, who were treated for precancerous cervical lesions using one of the following surgical procedures: LV, LLETZ or cold knife conization. The study design was approved by the national medical ethics committee. Each enrolled woman gave written informed consent.

Just before the surgical procedure, a cervical swab was taken with the Female Swab Specimen Collection Kit (Digene Corp., Gaithersburg, MD) and tested for the presence of high-risk HPV genotypes by the HCII test. The tests were done at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana. Only probes for the detection of high-risk HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,

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68, were used. The samples were analyzed according to the manufacturer's instructions. Results of the test were given as the ratio between relative light units (RLU) and the cut-off value. Specimens with RLU/cut-off value ratios of ≥ 1.0 were considered HPV positive for one or more high-risk HPV genotypes.

LV was done with a CO_2 laser (Sharplan 1030, Laser Industries LTD, Tel Aviv, Israel), using the power of 20 W, and LLETZ with an electro surgical unit (ERBOTOM ICC 200, ERBE Elektromedizin, Tübingen, Germany) operating at 200 W in high-cut mode. LV and LLETZ were done as outpatient procedures, whereas cold knife conization was done under general anesthesia.

Histological diagnosis was based on cone specimens obtained during LLETZ and cold knife conization, whereas in LV cases, the material was obtained by colposcopically directed biopsy of cervical lesions, done before LV.

The women who were HPV-positive before surgery were followed by the HCII test ten months after surgery. The women who were HPV negative before surgery, were followed by cytological testing only, and did not undergo further HPV testing.

In order to find whether the same HPV genotype was present before and after surgery, HPV genotypes were determined in both stored specimens of HPV positive women. HPV genotype determination was done by restriction fragment analysis of PGMY09/PGMY11 PCR products, as described previously [16, 17]. Briefly, DNA was extracted from the specimens, and the quality of each DNA sample was verified with the successful amplification of a fragment of the human beta globin gene. A 450 bp long part of the HPV L1 gene was amplified using PGMY09/PGMY11 consensus primers. Generated PCR products were digested using seven restriction endonucleases, and analyzed by agarose gel electrophoresis.

According to their oncogenic potential, HPV genotypes were divided into four groups [18]:

1) High-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82);

2) Probable high-risk HPV genotypes (26, 53 and 66);

3) Low-risk HPV genotypes (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108);

4) Undetermined risk HPV genotypes (25, 34, 57, 62, 74, 89 and HP 70).

Statistical analysis was done with the statistical package SPSS for Windows, Release 11.0, Standard version (SPSS Inc., Chicago, IL). One-way ANOVA test was used for determination of statistical significance. The differences were considered significant if p values were less than 0.05.

Results

The mean age of the 214 included women was 36.18 years (SD = 9.87); 61 (28.5%) women were younger than 30 years.

Histological diagnoses were as follows: 102 (47.7%) women had less than CIN 2 (< CIN 2), 41 (19.2%) women had CIN 2, 65 (30.4%) women had CIN 3 and four (1.9%) women had microinvasive carcinoma. In two (0.9%) women histological diagnosis was missing because the material was unsatisfactory for evaluation.

The HCII test before the procedure revealed that 114 (53.3%) women were positive for high-risk HPV genotypes. Of the women younger than 30 years, 38/61 (62.3%) were HPV-positive, and of the women aged 30 years or more, 46/153 (49.7%) were HPV positive (p = NS).

Cytological and histological diagnoses, and the percentages of HPV-positive women are summarized in Table 1.

Table 1. — *Cytological and histological diagnoses before surgical procedures (percentages of HPV-positive women in brackets).*

Cytology	Histology (% HPV-positive)				Total (% HPV (positive)
	< CIN 2	CIN 2	CIN 3	Microinvasive carcinoma	
Abnormal glandular cells	2 (0%)	0	2 (100%)	0	4 (50.0%)
Mild dyskaryosis	74 (16.2%)	21 (28.6%)	13 (76.9%)	0	108 (25.9%)
Moderate dyskaryosis	23 (60.9%)	18 (83.3%)	42 (88.1%)	1 (100%)	84 (79.8%)
Severe dyskaryosis	2 (50.0%)	2 (100%)	5 (100%)	3 (100%)	12 (91.7%)
Severe glandular dyskaryosis	1 (100%)	0	3 (100%)	0	4 (100%)
Total (% HPV positive)	102 (27.4%)	41 (56.1%)	65 (87.7%)	4 (100%)	212

Eighty-nine women (41.6%) were treated with LV, 111 women (51.9%) with LLETZ, and 14 women (6.5%) with cold knife conization. The procedures according to histological diagnoses and the proportions of HPV positive women are shown in Table 2.

Table 2. — *Surgical procedures according to histological diagnosis (percentage of HPV-positive women in brackets).*

Histology	LV (% HPV+*)	LLETZ (% HPV+*)	Conization	Total
< CIN 2	58 (27.6%)	42 (28.5%)	2 (0%)	102
CIN 2	16 (56.3%)	24 (58.3%)	1 (0%)	41
CIN 3	13 (84.6%)	41 (85.4%)	11 (100%)	65
Microinvasive carcinoma	0	4 (100%)	0	4
Total (% HPV+*)	87 (41.4%)	111 (58.5%)	14 (78.6%)	212

*% HPV+: Percentage of HPV positive women.

Of the 114 HPV positive women, 95 came for follow-up HPV testing. Five women were excluded because of hysterectomy done shortly after the initial surgery, and 14 women did not respond to our invitation to a follow-up HPV test. The hysterectomies were done in women older than 42 years: two after LLETZ-cone diagnosis of CIN 3 with positive margins, and three in women with microinvasive carcinoma.

The follow-up testing for HPV by HCII revealed that HPV infection was eliminated in 23/34 (67.6%) women treated with LV, in 44/51 (86.3%) women treated with LLETZ, and in 10/10 (100%) women treated with cold knife conization. The differences were statistically significant ($p < 0.05$).

HPV genotype determination was done in stored specimens from women who tested HPV positive before and after surgery. The results are shown in Table 3. The most frequently detected HPV genotype in our series was HPV 16, which was found to be present in 4/18 (22.2%) preoperative specimens, and in 3/18 (16.7%) postoperative specimens. The next most frequently detected HPV geno-

Table 3. — *Histological diagnoses and HPV genotypes before and after surgical procedures.*

No.	Age (yr.)	Histology	Surgery	Genotype at inclusion	Genotype after surgery
					Same genotype
1	22	Missing	LV	66‡, 56†	66‡
2	26	< CIN 2	LV	16†	16†
3	36	CIN 2	LLETZ	31†	31†
4	45	CIN 2	LLETZ	39†	39†
5	26	CIN 2	LV	18†	18†, 54§, 66‡
6	22	< CIN 2	LV	52†	52†, 61§
7	21	< CIN 2	LLETZ	31†	31†
8	73	CIN 3	LLETZ	45†	45†
9	31	CIN 2	LLETZ	33†	33†, 68†
10	35	< CIN 2	LV	45†	45†
11	29	CIN 3	LV	62‖, 51†, 89[5]	62‖
					Different genotype
12	26	CIN 3	LLETZ	45†	52†, 68†
13	26	< CIN 2	LV	16†	18†, X¶
14	21	CIN 2	LV	18†	66‡
15	37	Mic.inv. ca*	LLETZ	16†	68†
16	24	CIN 2	LV	18†	16†, 31†
17	29	CIN 3	LLETZ	16†	59†
18	29	CIN 3	LV	52†	16†

* Microinvasive carcinoma; † High-risk HPV genotype; ‡ Probable high-risk HPV genotype; § Low-risk HPV genotype; ‖ Undetermined risk HPV genotype; ¶ Uncharacterized HPV genotype.

types were 18, 45, 31, 52 and 66 (decreasing order of frequency). Persistent infection with the same and only one HPV genotype was found to be present in 8/18 (44.4%) specimens, the same HPV genotype plus an additional genotype was found in 3/18 (16.7%) specimens; infection with a different high-risk genotype was found in 6/18 (33.3%) specimens, and in 1/18 (5.6%) specimens infection with a different probable high-risk HPV genotype was detected.

Additionally, HPV genotype determination showed that in seven (38.9%) women a different HPV genotype was found to be present before and after surgery; these women acquired a new infection. Therefore, we calculated the corrected efficiency of the surgical procedures assuming that the women with a different HPV genotype before and after surgery were cured of the initial HPV infection. Thus, the effectiveness of LV, LLETZ and cold knife conization in eliminating HPV infection was even improved, and reached 79.4%, 92.7% and 100%, respectively (p = NS).

Discussion

Persistent infection with high-risk HPV genotypes is a necessary etiological factor for precancerous cervical lesions and cervical cancer [2, 3]. We wanted to establish the efficiency of LV, LLETZ and cold knife conization, done for precancerous cervical lesions, in eliminating high-risk HPV infection. LLETZ and cold knife conization are comparable as they both provide the material for histological analysis. On the other hand, LV is a technique that does not provide histological specimens, therefore colposcopically directed biopsies, done before surgery, were used for histological evaluation in this study.

Testing for HPV by HCII before the surgical procedure showed that 58.6% of women treated with LV, 41.5% of women treated with LLETZ and 21.4% of women treated with cold knife conization were HPV negative. In our study, the proportion of HPV negative women treated with surgery was too high and might indicate overtreatment, since women without high-risk HPV infection are not threatened by cervical cancer [2].

Histological diagnoses obtained from the specimens of women undergoing surgical treatment showed that the women with < CIN 2 were mostly treated with LV, and the women with CIN 2 or worse were mostly treated with LLETZ.

The HCII follow-up test, done in 95 women, revealed that cold knife conization was the most efficient in eliminating high-risk HPV infection. All women who were HPV positive before cold knife conization were HPV negative at follow-up. These outcomes are superior to those found by Bodner et al. [12] who registered a 73% eradication of HPV infection by cold knife conization.

After LLETZ, 86.3% of HPV positive women were HPV negative at follow-up. Kucera et al. [19] registered a 94% efficiency of LLETZ in the elimination of HPV infection. They completed the third follow-up test 12 months after surgery, thus we might speculate that some women in our study could become HPV negative within two more months. Houfflin Debarge *et al.* [20] showed a 63.2% clearance of HPV three months after LLETZ, which is poorer than in our study; this might be due to a shorter interval to the follow-up HPV testing (3 vs 10 months).

LV showed a moderate efficiency: 67.6% of HPV positive women were HPV negative at follow-up. In the available literature we could not find data on HPV persistence detected with the HCII test after treatment of precancerous cervical lesions with LV.

Data from the literature [10, 11, 21, 22] indicate that there is a high proportion of short-lasting, transient HPV infection in the population of young women. In our study, 28.5% of included women were younger than 30 years, and as many as 12/18 (66.7%) women, who were HPV positive after surgical treatment, were younger than 30 years. Using the PCR- PGMY09/PGMY11 method it was proven that a different HPV genotype after surgery was found present in 38.9% of HPV-positive women. Among the women with a different HPV genotype after surgical treatment, 6/7 (85.7%) were younger than 30 years.

HPV genotypes 16, 18 and 45 were most frequently detected in our study. These HPV genotypes are found in the majority of cervical cancer specimens [18, 23] and are also most prevalent in the general population worldwide [24]. Poljak *et al.* [17] recently found that the HCII test detects 15 HPV genotypes that are not included in its high-risk probe cocktail. Our study confirmed that the HCII test also detects HPV 66, which has been classified as a probable high-risk genotype [18]; additionally, HCII applied in our series was first found to detect HPV 62, which has been classified as undetermined risk HPV genotype [18].

After determination of HPV genotypes, we calculated the corrected efficiency of the surgical procedures assum-

ing that the women with different HPV genotypes detected before and after the surgical procedure were cured of the initial HPV infection. LV, LLETZ and cold knife conization were effective in eliminating HPV infection in 79.4%, 92.7% and 100%, respectively. This efficiency was better than that calculated on the basis of the HCII test alone, when it was 67.6%, 86.3% and 100%, respectively.

We agree with the authors who have recommended the HCII test to be used for follow-up after surgical treatment of precancerous cervical lesions, since it is simple and commercially available. The HCII test is a good complementary test to cytology, which has not been proven sufficiently effective in our country [25-27]. However, the follow-up HCII test detected seven HPV positive women who acquired a new infection as detected by the PCR - PGMY09/PGMY11. We are of the opinion that the HCII test can be used for the assessment of the efficiency of surgical procedures in eliminating high-risk HPV infection, especially in women aged 30 years or more. Additional HPV-genotype determination would improve reliability in women younger than 30.

In conclusion, the results of our study imply that the three analyzed surgical procedures are effective in eliminating high-risk HPV infection. Therefore, we recommend the usage of HPV testing at follow-up after treatment of precancerous cervical lesions since it can define a small proportion of women who need close surveillance, and possibly additional treatment.

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Address reprint requests to:
E. VRTAČNIK-BOKAL, M.D., Ph.D.
Department of Obstetrics and Gynecology
University Medical Centre Ljubljana,
Šlajmerjeva 3
SI-1000 Ljubljana (Slovenia)