

Human papillomavirus infection in patients with residual or recurrent cervical intraepithelial neoplasia

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ABSTRACT

Aims and background. The main purpose of this longitudinal study was to evaluate the frequency of HPV infection in patients with residual or recurrent CIN.

Methods. 797 consecutive patients with CIN, treated with conization, were included. In 38 patients with residual or recurrent CIN in whom reconization was performed, infection with high-risk HPV types was analyzed.

Results. Reconization was performed in 4.8% of patients. Before reconization, 21 patients (55.3%) were infected with high-risk HPV and 17 patients (44.7%) were HPV negative. Among the HPV-negative patients, two (11.8%) had CIN 1, five (29.4%) CIN 2, nine (52.9%) CIN 3 and one patient (5.9%) had microinvasive cancer of the uterine cervix. The difference in frequency of infection with high-risk HPV was not significant (chi-square 0.372; $P > 0.05$).

Conclusions. On the basis of the study results it is not possible to recommend the HPV test as the only method of detection of residual or recurrent CIN after conization.

Introduction

Cervical intraepithelial neoplasia (CIN) is a premalignant dysplasia of the cervical epithelium. At present a reliable prediction of which CIN will regress and which will progress is not possible, so every higher CIN grade (CIN 2 and CIN 3) is treated surgically to remove the altered tissue. Residual or recurrent neoplasia is found in 3-15% of women after CIN treatment¹⁻³. The factors most often associated with residual neoplasia and recurrence are the condition of the surgical cone margins, CIN extension to the cervical canal crypts, and the number of mitoses⁴. In cases where neoplastic tissue was present in the surgical specimen margins, the frequency of CIN recurrence was as high as 22%⁵. It has also been known for some time that effective CIN treatment is associated with the removal of high-risk HPV strains during the operation⁶. As high-risk HPV types are often found in CIN recurrences, the question of the role of HPV assessment after CIN treatment arises. Various studies report the presence of HPV after CIN treatment in 0-92%⁷. However, it is not always clearly stated whether these are nonradical resections, CIN presence immediately following surgery, or actual disease recurrences. Frequently the studies also differ in surgical method, so their comparison can only serve as a frame of reference.

Because of the lack of data on HPV presence in women from northeast Slovenia in whom repeated CIN treatment is required, we analyzed the frequency of infection with high-risk HPV types in patients treated at Maribor University Hospital who required reconization. To this aim we included the patients with CIN persisting after primary treatment as well as those with later recurrences.

Key words: human papillomavirus, infection, CIN, residual lesion, recurrence, reconization.

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Patients and methods

On the basis of prospectively collected data of the Department of Gynecologic Oncology and Oncology of the Breast at Maribor University Hospital we analyzed infection with high-risk HPV strains in CIN patients in whom reconization had been carried out between 1 January 1999 and 31 December 2004. The study sample was derived from a larger group of 797 women subjected to cervical conization, of whom 38 recurred. All patients included in the study signed an informed consent form and the study protocol was approved by the local Ethics Committee.

The study patients were between 29 and 70 years old (mean \pm SD 39.9 \pm 8.1 years). Between 1 and 336 months elapsed from conization to reconization (mean \pm SD 57.9 \pm 79.3 months). In the 1999-2002 period, *in situ* hybridization was used to determine HPV types (26 patients), after 2002 the Hybrid Capture technique HC II was used (12 patients).

Samples for HPV detection were taken immediately before reconization. Before the procedure, colposcopy had been performed in all study subjects. All reconizations were performed by cold-knife excision of the cone and by electrocoagulation of the cone excision site.

Results

In the 6-year observation period, 797 conizations and 38 reconizations (4.8%) were performed. The numbers of conizations and reconizations included in the study in the observation period are presented in Table 1.

Reconization was most often performed in patients with CIN 3 on conization (in 31 patients or 81.6%). For CIN 2 it was performed in 7 cases (18.4%). The most frequent post-reconization histopathological finding was CIN 3 (65.8%); CIN 2 and CIN 1 were found in 6 cases each (15.8%), and microinvasive cancer in 1 case (2.6%). The frequency of various grades of CIN after conization and reconization is presented in Table 2.

The frequency of HPV 16, 18, 31 and 33 infection, determined by *in situ* hybridization, at various CIN degrees prior to reconization is presented in Table 3. Among 13 patients infected with HPV, 7 were infected

Table 1 - Conizations and reconizations included in the study between 1999 and 2004

Year	Conizations (n)	Reconizations (n)	%
1999	123	6	4.9
2000	117	8	6.8
2001	139	6	4.3
2002	109	7	6.4
2003	123	3	2.4
2004	186	8	4.3
Total	797	38	4.8

Table 2 - Histopathological results by CIN grade after conization and reconization

Conization	Reconization N (%)				Total
	CIN 1	CIN 2	CIN 3	Invasive cancer	
CIN 1	0	0	0	0	0
CIN 2	0	3 (7.9)	3 (7.9)	1 (2.6)	7 (18.4)
CIN 3	6 (15.8)	3 (7.9)	22 (57.9)	0	31 (81.6)
Invasive cancer	0	0	0	0	0
Total	6 (15.8)	6 (15.8)	25 (65.8)	1 (2.6)	38 (100.0)

Table 3 - HPV 16, 18, 31 and 33 infection in 26 patients with CIN before reconization (*in situ* hybridization)

CIN grade	HPV status (<i>in situ</i> hybridization) N (%)		Total
	HPV 16, 18, 31, 33 negative	HPV 16, 18, 31, 33 positive	
CIN 1	2 (7.7)	4 (15.4)	6 (23.1)
CIN 2	2 (7.7)	0	2 (7.7)
CIN 3	8 (30.8)	9 (34.6)	17 (65.4)
Invasive cancer	1 (3.9)	0	1 (3.9)
Total	13 (50.0)	13 (50.0)	26 (100.0)

with only 1 strain of HPV and 6 with 2 strains. None of the patients was infected with 3 or 4 HPV strains. The frequency of infection with high-risk HPV as determined by HC II in 12 patients is shown in Table 4. Table 5 shows that prior to reconization nearly half of the patients were HPV negative, while slightly more than half showed infection with high-risk HPV types.

Table 4 - High-risk HPV infection at various grades of CIN before reconization in 12 patients (HC II)

CIN grade	HPV status (HC II) N (%)		Total
	HC II negative	HC II positive	
CIN 1	0	0	0
CIN 2	3 (25.0)	1 (8.3)	4 (33.3)
CIN 3	1 (8.3)	7 (58.3)	8 (66.7)
Invasive cancer	0	0	0
Total	4 (33.3)	8 (66.7)	12 (100.0)

Table 5 - High-risk HPV infection in patients with CIN before reconization

CIN grade	HPV status N (%)		Total
	HPV negative	HPV positive	
CIN 1	2 (5.3)	4 (10.6)	6 (15.8)
CIN 2	5 (13.1)	1 (2.6)	6 (15.8)
CIN 3	9 (23.7)	16 (42.1)	25 (65.8)
Invasive cancer	1 (2.6)	0	1 (2.6)
Total	17 (44.7)	21 (55.3)	38 (100.0)

In the entire observed group of 38 patients, prior to reconization 21 patients (55.3%) were infected with high-risk HPV and in 17 patients (44.7%) HPV infection could not be confirmed. The difference in the frequency of infection in patients with high-risk HPV types was not significant (chi-square 0.372; $P > 0.05$).

Discussion

In 2003 Dannecker *et al.* published the results of an 18-study meta-analysis of the presence of HPV infection after CIN treatment⁸. Most studies showed that HPV disappears after successful treatment of CIN.

Many studies reported an extremely low HPV persistence after successful treatment of CIN⁹⁻¹². Cruickshank *et al.* found HPV in 12% patients without recurrence and in 29% patients with CIN recurrence¹³. On this basis they calculated that women with persistent HPV after CIN treatment have a 2.9-times higher risk of disease recurrence. Likewise, Acladios *et al.* established the presence of HPV in 47% patients with CIN recurrence and in only 6% of those without CIN recurrence¹⁴. Kjellberg *et al.* identified the presence of high-risk HPV in only 2.7% patients within 35 months after conization¹⁵. At the same time they found a high percentage of HPV presence in 37 patients who had not received treatment. This proves that virus clearance is an actual consequence of CIN treatment and not a coincident event. Tjong *et al.* proved that HPV transmission and the resulting infection are local events in the lower genital tract, and are subject to local immunological mechanisms¹⁶.

Although various authors established the presence of HPV infection in patients with CIN in different countries, it is common knowledge today that HPV infection represents an indispensable yet insufficient condition for CIN development. In our study reconization had to be done in 4.8% of cases, which corresponds to data from the literature^{1,2}. Reconization was required particularly in those patients whose cone histopathology findings revealed a higher grade of dysplasia (CIN 2 and CIN 3). Reconization was not performed in any case in which conization revealed CIN 1. As early as 1993, Guijon *et al.* discovered that failure of CIN therapy was associated with HPV infection¹⁷. Chua and Hjerpe attempted to assess the significance of HPV as a prognostic marker following conization. They came to the conclusion that recurrences can be the consequence of persistent neoplastic changes or not fully cleared subclinical HPV infections. They also stated that HPV testing could be applied in monitoring the success of conization⁷. Our study ascertained infection with high-risk HPV in 21 patients (55.3%), so we concluded that HPV testing following conization as a method to complement cytological investigation of the cervix and colposcopy is not justified. Kaufman and Adam studied the

significance of HPV typing in the triage of women with low-grade cervical cytological abnormalities. They realized that for the time being this test cannot be used in regular clinical practice¹⁸. Murta *et al.* studied the frequency of neoplasia involving the surgical margins. Their study comprising 201 conizations showed that 26.4% of cone margins were affected by neoplasia¹⁹. Bretelle *et al.* studied the success (radicality) of operative procedures in CIN therapy. They found that excision of a cone with involved margins is not a reliable sign of residual disease, and so in such cases reoperation is not always essential²⁰. The absence of high-risk HPV strains in nearly half (44.7%) of our study patients with reconization can be explained by the limited spectrum of oncogenic HPV determination in test samples as well as by the fact that there is the possibility of dysplasia and cervical cancer developing in the absence of HPV²¹. Moreover, we should be aware of the possibility of inadequacies in obtaining and processing samples.

Due to the lack of prospective randomized studies dealing with the importance of assessing the presence of HPV infection following CIN treatment, no final conclusions are as yet possible. Nevertheless, it is clear that successful treatment of CIN is followed by the disappearance of HPV from the cervical region. We should be fully aware that the presence of HPV infection is a prerequisite for CIN development or recurrence²²⁻²⁶. CIN is currently treated by removing or destroying the neoplastic cervical tissue. Data on HPV infection are also important when choosing the therapeutic method since there is evidence that complete clearance of HPV from the cervix is less reliable with certain types of therapy (cryotherapy)²⁷.

A negative test for the presence of HPV following CIN treatment has a high negative predictive value, as it excludes the presence and recurrence of CIN with great probability. This is particularly important in case of changes removed *non in sano* since, on the basis of a negative HPV test, negative cytology and colposcopy results, we can protect such patients from a repeated operative procedure (reconization). However, we must consider the fact that HPV clearance from the cervix may take several months. In view of known data it is not recommended to carry out the HPV test earlier than 6 months after CIN treatment. It is understood that we must not disregard cytological investigation of the cervix²⁸.

In our study population the prevalence of high-risk HPV types in women with residual or recurrent CIN was 55.3%. On the basis of our study results we cannot recommend the HPV test as the only method of detecting residual or recurrent CIN following conization.

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