Laser vaporization in the management of CIN

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Summary

Aims: To evaluate the effectiveness of laser CO_2 vaporization in high-grade cervical intraepithelial neoplasias and to assess the diagnostic reliability of cytology, colposcopy, microbiology and HPV tests in predicting recurrence in a long-term outcome. *Methods:* Forty-four patients affectd by high-grade cervical intraepithelial neoplasia (HG-CIN) were submitted to laser CO_2 vaporization and followed-up a minimum of five years. Vaginal smears for microbiological examination were detected. HPV testing was performed by polymerase chain reaction (PCR). *Results:* The average age of the patients was 19.5 years (range 15-24). The cure rate after a single treatment was 95%. Two cases (5%) revealed HG-CIN persistence after three months. The five year follow-up of all cases submitted to a second laser procedure revealed negative cytologic and colposcopic findings. *Conclusions:* A higher degree of expertise and experience from the colposcopist and long-term follow-up proves the effectiveness of laser vaporization in the management of CIN in young women. It has been suggested that HPV infection alone may not be sufficient to promote carcinogenesis and that other cofactors could be involved. Microbiological tests are important to identify and treat any inflammation which might represent a cofactor of HPV infection in the pathogenesis of cervical dysplasia. Cytocolposcopic long-term follow-up, microbiological and HPV tests can improve regression of disease.

Key words: Cervical intraepithelial neoplasia; HPV; Recurrence; Adolescents.

Introduction

Cancer of the uterine cervix is the second most common cancer among women worldwide, with approximately 493,000 new cases and 274,000 deaths in 2002 [1].

The casual role of HPV in all cancers of the uterine cervix has been firmly established biologically and epidemiologically [2].

High-risk HPV (HR HPV) infections seem to persist longer than low-risk infections [3].

During reproductive age, if a cytological high-grade squamous intraepithelial lesion (HG-SIL) is detected, conservative treatment is mandatory to eradicate the lesion and to preserve reproductive function [4]

Often excisional methods have also been chosen as the treatment for ectocervical high-grade cervical intraepithelial neoplasias because of the significant incidence of microinvasion in excised specimens and reported cases of invasive disease following local destructive procedures [5-8].

The advantages of carbon dioxide laser vaporization in treating CIN have been outlined, but most studies have not provided long enough follow-up periods to acquire sufficient information on recurrence rate and disease progression [9-12].

A skilled colposcopist with cyto-colposcopic follow-up is indicated after conservative treatment of CIN [13-16].

Persistence of HPV is associated with an increased risk of developing cervical dysplasia and cancer. Women who test positive for HPV persistently over time have been shown to be at the highest risk of developing preneoplastic genital disease [17].

It has been suggested that HPV infection alone may not be sufficient to promote cervical carcinogenesis and that other cofactors could be involved [2].

Microbiological tests in abnormal cytology are important to identify and treat any inflammations, even if asymptomatic, which might represent a cofactor of HPV infection in the pathogenesis of cervical intraepithelial lesions [18].

The objective of this study was to evaluate the effectiveness of CO_2 laser vaporization of high-grade cervical intraepithelial neoplasia (HG-CIN) in adolescents and to assess the diagnostic reliability of cytology-colposcopy, microbiological and high-risk HPV testing, improving relapse disease in long-term follow-up.

Materials and Methods

Forty-four patients with abnormal cervical cytology classified as HG-SIL, formulated in agreement with the Bethesda System [19] and histologically confirmed, were recruited.

The patients were interviewed about personal history (age, parity, sexual habits, referred tobacco use, oral contraceptive use, previous cervical treatments and past genital infections) and gave their informed consent.

Microbiological testing was performed by vaginal smears through microbiological cultures and by fresh bacterioscopic examination.

Molecular detection of HR HPV was performed by PCR.

Cytological samples were collected in sterile 1.5 polypropylene tubes and resuspended in 100 µl of digestion buffer with proteinase K, incubated overnight at 37°C, and boiled for 5 min. Aliquots (10 µl) of each were used for PCR amplification. Each cytological sample was analyzed by PCR for HPV open reading frame sequences using the following primers: HPV-16, 5'-ACC gAA ACC ggT Tag TATAAAAgC-3' and 3'-gAT CAT TTg TCT CTg gTT gCA AAT-5'; HPV-18, 5'-CAC ACC ACA ATA

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CTA Tgg CgCgCT-3' and 3'-CTg CTg gAT TCA ACg gTT TCT ggC-5'. Every amplification experiment included one negative and one positive control for each viral type. A portion of exon 15 of the human APC gene was routinely amplified as a positive control using the following primers: APC, 5'-gTCCTTCACAgAAtgAAAgATg-3' and 3'-CTg CTT gAA gAA gAC ATA TgTTCg-5'.

The sizes of the amplified fragments were 576, 360 and 520 bp, respectively. Amplification reactions were carried out in 100 µl of reaction buffer containing 50 mM KCl, 2 mM MgCl2, 10 mM Tris (pH 8.3), 200 µM each deoxynucleotide triphosphate, 2.5 units of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 100 pmol of each primer, and 10 µl of proteinase K-digested sample. Samples were denatured at 95°C for 5 min, followed by 40 cycles of amplification (denaturation at 94°C for 1.5 min, annealing at 55°C for 2 min, except APC, where annealing was at 40°C and 57°C, respectively, and extension was at 72°C for 2 min; the final extension was prolonged to 7 min).

Amplified products (15 µl) were electrophoresed through 1.6% agarose gels. The gels were analyzed by UV after staining with ethidium bromide [20].

The patients underwent colposcopy (Zeiss OM 50 colposcope. Carl Zeiss Inc; Germany) using a 5% acetic acid solution followed by the Lugol test and colposcopic findings were interpreted according to the International nomenclature [21].

Topography and the size of ectocervical lesions were determined by colposcopy and the number of quadrants involved by the abnormal transformation zone (ANTZ) were recorded dividing the cervix into four quadrants (1 quadrant or 25% of the cervix, 2 quadrants or 25-50% of the cervix, 3 quadrants or more than 50% of the cervix, 4 quadrants or more than 75% of the cervix).

The patients whose colposcopic examination detected completely ectocervical lesions and an entirely visible squamo-columnar junction (SCJ) and no more than three quadrants of cervix involved by lesions were considered available for treatment.

Laser vaporization was performed using a laser CO_2 Coherent System 451, connected to a Zeiss OM50 colposcope (Carl Zeiss Inc; Germany); surgical procedures were carried out in day surgery, under colposcopic guidance, without local anesthesia. The mean ablation depth was 7 mm (range 6-8 mm). Laser vaporization was performed in patients whose cytology, colposcopy and histology were in agreement.

The patients were followed-up by cytology and colposcopy every three months for the first year, every six months for the second year and then annually for a minimum of five years.

In any suspicious area of persisting or relapsing lesion, a colposcopic directed biopsy was performed.

In patients with histologically confirmed relapsing of HG-CIN a large electrosurgical excisional procedure (LEEP) was carried out.

The median follow-up was 72 months (range: 60-120 months).

Results

Histological analysis of colposcopy-directed biopsies revealed CIN2 in 11 out of 44 (25%) patients and CIN3 lesions in 33 out 44 (76%) patients.

The average age of patients was 19.5 years (range: 15-24), mean parity 1.7 (0-5), mean age for first sexual intercourse 16 years (14-24), and the mean number of sexual partners four (1-10); 52% referred tobacco use, 32% previous cervical treatment for clinical HPV lesions or CIN and 74% for past genital infections.

Table 1. — *Cytology, histology and HPV test results*.

Initial cytology	Histology	HPV test	
No. of patients (%)		pos 16 /	pos 16-18
High SIL	CIN2 11 (25%)	11	–
	CIN3 33 (75%)	29	4
Total 44		40	4

SIL = squamous intraepithelial lesion; CIN = cervical intraepithelial lesion.

In every patient pretreatment colposcopy visualized an entirely detectable SCJ and an ectocervical ANTZ.

Six (13%) ANTZ cases showed grade 1 of abnormality and 38 (87%) revealed grade 2.

Cumulative failure rate at first treatment was 5% and where the lesion involved three quadrants the percentage was 4%.

Microbiological examination was negative in 11 (26%) for vaginal secretion and positive in seven (15%) for mycetes, in four (8%) for *Gardnerella vaginalis,* in six (13 %) for *Trichomonas vaginalis,* in eight (16%) for *Chlamydia tracomatis,* in three (6%) for Trichomonas and Gardnerella, in two (5%) for Streptococcus agalactiae, in three (6%) for Enterococci and in two (5%) for Cocchi.

The patients were submitted to proper local and systemic therapy before starting any surgical treatment.

HPV testing resulted positive for HPV type 16 in 40 cases (91%) and for HPV types 16-18 in four (9%). No early or late complications were observed.

The patients fully returned to normal activities within four to six weeks of treatment.

Forty-two (91%) patients had negative cytology and colposcopy at the first year follow-up check.

In two patients (5%) HG-CIN recurrences were observed.

Detectability of SCJ after treatment was considered the parameter defining a satisfactory colposcopic follow-up; in 43 treated cases (97%) the SCJ was entirely visible. In one (3%) it was partially endocervical and visible only after dilation of the cervical canal.

At one-year follow-up seven (16%) patients were positive for HR HVP testing but they had negative cytology.

At two-year follow-up four (9%) patients were positive for HR HVP testing and they also had negative cytology.

The 5-year follow-up of all cases submitted to a second laser procedure revealed negative cytological and colposcopic findings. All had complete regression of cytological abnormalities within a year of treatment.

Discussion

Persistence of HPV is associated with an increased risk of developing SIL and cancer. In younger women, it has been shown that persistent viral detection represents a more accurate measure of risk for development of cervical neoplasia than do tests taken at a single point in time.

Several prospective studies have shown that women

who are HPV-DNA-positive at baseline have a higher risk of developing CIN3 or invasive cervical cancer during the follow-up than HPV-DNA-negative women [2].

In our series the 5-year failure rate was 18% (percentage of treated patients with absence of recurrent/persistent HG CIN). A single destructive treatment case of invasive carcinoma occurred after a mean follow-up of six years.

Increasing grade of CIN has been identified as an important factor in failure rates but a correlation with grade of CIN has not been found indicating that the size of lesion is more important.

Patients with one quadrant involved had a recurrence rate of 2% (1 case), those with two quadrants involved 5% (2 cases), and those with more than two quadrants involved 9% (4 cases) [22].

Ferenczy [23] reported a 29% failure rate with CIN3, and in those patients whose lesion was greater than 30 mm in size, the failure rate was 38% [23].

Laser vaporization represents a low-morbidity procedure because there are no intra- or postoperative complications [11, 24].

Available cytocolposcopic follow-up is one of the most important reasons to choose an ablative procedure as treatment.

Patients should be examined by an experienced and skilled colposcopist because the most important factor determining the quality of clearance results is the diagnostic accuracy [22].

If colposcopy is satisfactory, the procedure is known to reveal residual disease in the presence of negative cytology. A satisfactory colposcopic follow-up was possible in 99.4% of patients, permitting early diagnosis of persistence.

It is believed that HPV type affects both the absolute risk of viral persistence and progression to dysplasia given viral persistence. HPV 16 appears to be remarkably carcinogenic with an absolute risk of CIN3 approaching 40% at five years of persistence [24].

Persistence of HPV is associated with an increased risk of developing dysplasia and cancer.

Women who test positive for HPV persistently over time have been shown to be at the highest risk of developing preneoplastic genital disease [17].

It has been suggested that HPV infection alone may not be sufficient to promote cervical carcinogenesis and that other cofactors could be involved, such as cigarette smoking, oral contraceptives, immunosuppression, vitamin deficiency, and other sexually transmitted diseases [25-27].

Sexually transmitted diseases have been considered as possible cofactors in the pathogenesis of carcinoma of the uterine cervix, even if no single agent has been identified as particularly significant [28].

The role of *Chlamydia trachomatis* (CT) infection as a risk factor in the development of cervical lesions is controversial. Some authors suggest that combined infection with HPV plays a central role in the etiology of intraepithelial lesions of the uterine cervix and represents a risk for the subsequent development of invasive cervical neoplasia when associated with other factors, such as cigarette smoking and sexual promiscuity [26, 27, 29-31]. However, other authors suggest that infections due to HPV or CT are independent [32].

Other authors [33, 34] found that women with cervical cytological abnormalities presented higher frequencies of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection.

Patients who present abnormal Pap tests should undergo cervicovaginal microbiologic examinations for potential pathogens, especially before any treatment of SIL, and even more so in persistent or recurrent cases of papillomavirus infections [33].

Therefore abnormal cytology is relevant in identifying and treating any inflammations, even if asymptomatic, which might represent a cofactor of HPV infection in the pathogenesis of cervical intraepithelial lesions.

Available cyto-colposcopic long-term follow-up in selected cases, after destructive procedures, for HG-CIN could be combined with microbiologic and HPV testing which might represent cofactors involved in persistence, recurrence and progressive disease.

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