Human Papillomavirus DNA in CO_2 Laser-Generated Plume of Smoke and its Consequences to the Surgeon

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Carbon dioxide laser energy is absorbed by intracellular water but not by proteins or nucleic acids. The possibility of dispersing viral DNA during laser therapy of human papillomavirus (HPV)-containing genital infections was explored using a filter hybridization technique. Samples were taken using dacron swabs from 110 patients in nine separate treatment sessions as well as from five pre-filter canisters, four fume vacuum tubes, and from the nasopharynx, eyelids, and ears of the laser surgeon before and after laser surgery. The viral RNA probes were specific for groups of HPV types 6/11, 16/18, and 31/33/35. Human papillomavirus DNA was identified in swabs from 65 of 110 (60%) of histologically unequivocal condylomata and cervical intraepithelial neoplasias. One of the five pre-filter canisters (20%) tested was HPV DNA-positive after laser treatment of 65 patients; it contained HPV DNA type 6. The four fume vacuum tubes tested in the remaining 45 patients were HPV DNA-negative, as were the nasopharynx, eyelids, and ears of the operator. Although HPV DNA may be released during laser vaporization of genital HPV infections, contamination of the operator is unlikely provided appropriate equipment for evacuating HPV DNA-positive smoke is used. *(Obstet Gynecol 75:114, 1990)*

Among the many treatment modalities available for human papillomavirus (HPV)-containing genital lesions, CO_2 laser therapy has become one of the most attractive techniques used in current gynecologic practice.[1] Carbon dioxide laser energy is largely absorbed by intracellular water, which is instantaneously steamed and results in the vaporization of lased cells. However, intracellular proteins and nuclear DNA are water-free and are thus carbonized rather than vaporized. The resulting plume of smoke contains charred and thermocoagulated cellular debris[2] which is evacuated from the treatment field by suction. Previous analyses failed to indicate viability of the carbonized particulates, including those derived from normal and malignant cells as well as those from verruca and bacteria.[2–4] Recently, however, papillomavirus DNA was found by Southern blot hybridization in the plume of smoke from cutaneous bovine fibropapillomata and from human plantar warts.[5] It is not known whether CO_2 laser treatment of genital warts is associated with HPV DNA-contaminated fumes. Concern has been raised that such a possibility may exist and that if so, then the mucous membranes of the nasopharynx of the operator, the operating room personnel, and the patient may be at risk of plume-generated HPV infection.

The aim of this study was to explore, with the aid of molecular hybridization techniques, whether laser treatment of HPV-containing genital infections is associated with viral dispersion.

Materials and Methods

One hundred ten patients, 97 women and 13 men, aged 16–55 years old (mean 32) with histologically diagnosed HPV infections of the lower genital tract were vaporized with a Merrimack 840 CO_2 laser (Merrimack NIIC, USA, Inc., Redwood City, CA). The lesional tissues consisted of ordinary condylomata acuminata in 75 patients and flat, acetowhite condylomata in the remainder. The vast majority of the former lesions (95%) were located on the external anogenital skin, whereas the majority of the latter (95%) were located on the cervix. The lesional areas treated ranged from 2–15 cm^2 (mean 6). Laser vaporization was carried out in nine different treatment sessions, each including 11–13 patients. The minor operating room of the Reddy Memorial Hospital was used for laser vaporization of lesional tissues in 40 and 70 patients under

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general and local anesthesia, respectively. Power densities used ranged between 500–2000 W/cm^2, and laser energy was delivered in a continuous or intermittent pulse mode. The operating room contained a wall-mounted exhaust system for evacuating air from inside out. The laser-generated plume of smoke was evacuated with the Stackhouse Point One System (Stackhouse Association, Inc., El Segundo, CA) laser smoke filtration system using vacuum suction settings between moderate and high (6 and 9) and by placing the distal end of the vacuum tube about 1–2 cm from the treatment field. For lesions of the cervix and vagina, we used a commercially available, black rough-surfaced speculum with a smoke evacuation metal cannula welded on the inner surface of the upper blade. During each laser session, all operating room personnel wore standard surgical masks and commercially available protective plastic eyeglasses.

In all patients immediately before treatment, cellular samples for filter hybridization (Virapap/Viratype; Life Technologies Inc., Gaithersburg, MD) were obtained using saline-moistened dacron swabs that were rubbed firmly against the surfaces of the lesional tissues. Similarly, samples were taken from the fume in-flow end of the disposable pre-filter canister and from the inner surface of the distal end of the disposable vacuum tube. New pre-filter canisters and vacuum tubes were used for each of the nine treatment sessions. The nasopharynx, eyelids, and external auditory canal including the eardrum, but not the hands of the laser operator (AF), were also tested for HPV DNA. Cellular sampling for HPV detection and typing was repeated from the above objects and structures after the end of each laser treatment session. In the first part of the study, we tested only the material from the plume that had accumulated on the inner surface of the distal end of the vacuum tube (group A). This part of the study involved 45 patients treated in four different sessions. The second part of the study tested only the pre-filter canisters and involved 65 patients (group B) treated in five different sessions.

The filter hybridization was performed using reagents and methods contained in the ViraPap HPV DNA Detection Kit (Life Technologies Inc.). The collected exfoliated cells were transported using the ViraPap HPV Specimen Collection Kit. The samples were then incubated with Sample Preparation Reagent for 1 hour at 37C. After cell lysis, 250 μL was removed and transferred to new tubes. These samples were treated with Sample Diluent to achieve DNA denaturation, applied to a nylon membrane in a 25-well ViraPap Filter Manifold, and filtered under vacuum. Immediately after complete filtration, the membrane was transferred to a reaction tray, covered with the Prehybridization Reagent, and incubated at 60C for 30 minutes. After this incubation, the Prehybridization Reagent was thoroughly absorbed off the membrane and the Hybridization Reagent was added. This solution contains a mixture of full-length ^{32}P-labeled RNA transcripts homologous to HPVs 6, 11, 16, 18, 31, 33, and 35 DNA. The hybridization was carried out at 60C for 2 hours. After hybridization, the membrane was washed to remove the Hybridization Reagent, treated with RNase to remove nonspecifically bound probe, and then washed at 60C under high-stringency conditions. The membranes were then sealed in bags and exposed to Kodak X-omat AR x-ray film with intensifying screens at −70C. The length of the autoradiographic exposure depended on the age of the kit.

The ViraType HPV DNA Typing Kit (Life Technologies Inc.) applies the same procedures described for the ViraPap HPV Detection Kit, with the following exceptions. After cell lysis, 450 μL was removed and treated with Sample Diluent to achieve DNA denaturation. The samples were then applied in equal aliquots to three nylon membranes and filtered under vacuum. After pre-hybridization, the membranes were hybridized with three separate probe mixes specific for HPV types 6/11, 16/18, or 31/33/35. The probes have been made against the whole length of the HPV genome. The hybridization conditions, all remaining wash steps, and autoradiography were the same as those described for ViraPap.

We compared the accuracy of these tests for detecting HPV DNA with the standard reference method of Southern blotting. Specimens from 830 patients were collected and aliquots were analyzed by ViraPap and Southern blotting. The ViraPap test had a sensitivity of 94.5% and a specificity of 95.5% (S. Challberg, Life Technologies Inc., personal communication). In a similar analysis of 262 patient specimens, the ViraType test had a sensitivity of 97.4% and a specificity of 95.7% compared with Southern blotting (S. Challberg, Life Technologies Inc., personal communication). The limits of detection of these tests are approximately 3, 1, and 3 pg for ViraPap, ViraType, and Southern blotting, respectively.

Results

We tested 110 cases, 56 of which were histologically typical condylomata acuminata and 54 of either flat condylomata (plana) or cervical intraepithelial neoplasia. Sixty-five of the 110 cases tested (60%) were HPV DNA-positive. Human papillomavirus DNA positivity was higher in acuminate (40 of 56, 71%) than in flat condylomatous lesions and intraepithelial neoplasia (25 of 54, 46%), and was also higher in the 41 external

"J10C/G34C"

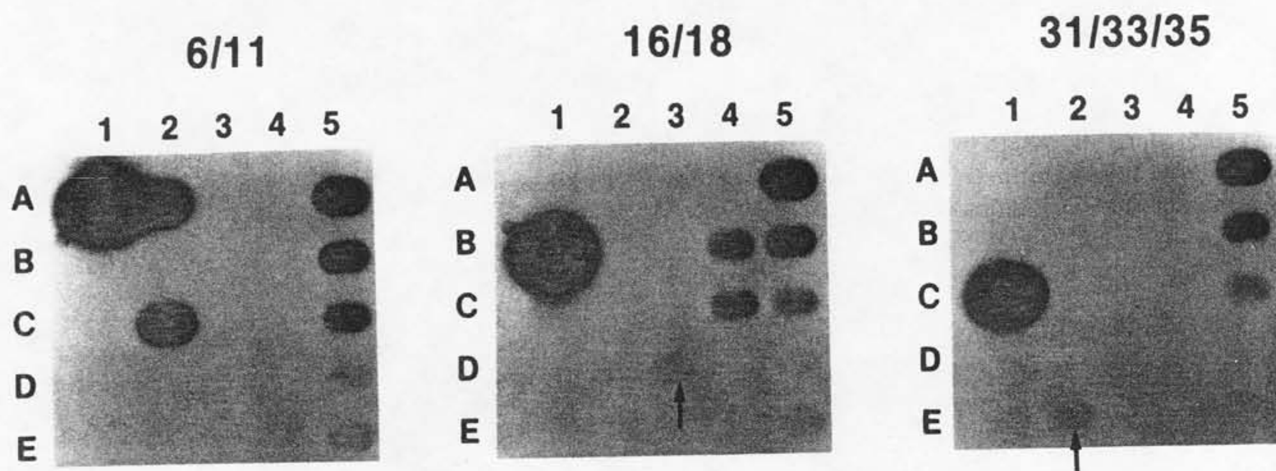

Figure 1. Representative ViraType runs for HPV types 6/11, 16/18, and 31/33/35. Positive controls are found in 1A, 1B, and 1C in the three panels, respectively. Lane 5 represents serial control dilutions of HPV DNA ranging from 10 to 0.5 pg in each panel. Positive results are found in 2A and 2C (6/11 panel); 3D (*arrow*), 4B, and 4C (16/18 panel); and 2E (*arrow*) (31/33/35 panel).

anogenital lesions (63%) than in the 24 cervical and vaginal lesions (37%).

None of the samples obtained before treatment from the operator, the five filters, and the four vacuum tubes tested positive for HPV DNA. Among the 45 group A patients, 28 (62%) had HPV DNA-positive lesions, but HPV DNA was not found in the vacuum tubes after vaporization. The rate of HPV DNA positivity in group B lesions was 37 of 65 (54%), and one of the five pre-filter canisters (20%) exposed to the smoke plume tested positive for HPV DNA type 6 (Figure 1).

Of the 65 cases in which the five filters were used, a similar number of lesions was tested in the internal (32 cases of cervical/vaginal) and external (33 cases of vulvar/anal/penile) lower genital tract. In addition, about the same number of flat (33 cases) and acuminate (32 cases) lesions was encountered.

Table 1 presents the location and colposcopic appearance of HPV-positive lesions in group B patients. The majority of HPV-positive lesions (22 of 37) contained types 6 and 11, were located on the external anogenital skin (19 of 37), and were of the acuminate condyloma type (19 of 37). We detected HPV positivity more frequently in lesions located on the vulva/anus/penis (23 of 33) than in those in the cervix and vagina (14 of 32). Similarly, acuminate lesions tested positively for HPV DNA more frequently (21 of 32) than did their flat counterparts (16 of 33). None of the cellular samples obtained from the operator after each of the nine laser treatment sessions tested positive for HPV DNA.

Discussion

From this study, it is clear that CO_2 laser therapy of HPV infections may disperse viral DNA. This was evidenced by the presence of HPV DNA in the CO_2

Table 1. Location and Colposcopic Appearance of Human Papillomavirus-Positive Lesions in Group B Patients (*N* = 37)

	HPV types		
	6/11	16/18	30s*
Location			
Cervix/vagina	3	5	6
Vulva/anus/penis	19	4	0
Total	22	9	6
Colposcopic appearance			
Flat	3	7	6
Acuminate	9	2	0
Total	12	9	6

HPV = human papillomavirus.
* Includes HPV types 31, 33, and 35.

laser-generated plume of smoke captured by the pre-filter of a commercially available smoke evacuation system. The results confirm earlier experiments on bovine fibropapillomata and human plantar warts.[5] However, HPV DNA positivity of the pre-filters in our study was comparatively lower than in the study of Garden et al.[5] In the latter, laser smoke was HPV DNA-positive by Southern blot hybridization[6] from four of four and two of seven bovine fibropapillomata and plantar warts, respectively. In the present study, only one (20%) of the five pre-filters tested for HPV DNA was positive for HPV type 6 by ViraPap. The design for the HPV DNA collection technique, the viral content of the respective lesions lased, and the different laser treatment modalities used probably account for the discrepancy in the incidence of HPV DNA positivity between the studies.

In the study of Garden et al,[5] the plume of smoke was collected in a specifically designed phosphate-buffered saline bubble-chamber apparatus installed in a vacuum system of 500 mg mercury. Such a collection technique may trap and preserve considerably more viral DNA than the routinely used pre-filter canister. The canister lacks an appropriate collection medium for viral DNA and is located three to six feet from the treatment field. Although we were unable to demonstrate viral DNA in the carbonized-oily, thermocoagulated deposit in the distal end of the vacuum tubes, it is possible that HPV DNA is preferentially carried in the lighter, smoky vapor and is deposited further along the wall of the tube. This may result in a lesser amount of viral DNA captured at the filter level. In addition, the position of the vacuum tube, about 1–2 cm from the treatment field, might have contributed to the relatively low HPV DNA positivity of the pre-filters in this study. Indeed, according to our experience, a large amount of nonvaporized material is released in the air when the distal end of the vacuum tube is positioned 2 cm from the lased area. Although this possibility may exist, the fact that the operator tested negative for HPV DNA after nine different treatment sessions over a 5-week period suggests that viral dispersion is limited to the immediate surroundings of the treatment fields.

Bovine fibropapillomata and human plantar warts contain considerably more HPV DNA per cell than do human genital warts.[7] They also contain more intact HPV DNA than do genital warts. This could explain the high yield of viral DNA in the laser smoke found by Garden et al.[5] Hybridization techniques cannot distinguish between HPV DNA and intact, fully formed, and potentially infectious virus. However, based on the very low number of fully formed virions in genital HPV infections, their presence in laser-generated smoke is less likely.

The type and mode of CO_2 laser energy delivered to vaporize genital HPV infections appears to play little role in HPV DNA dispersion. In our study, we used intermittent to continuous exposures with focused and defocused beams, and power densities that ranged from 500–2000 W/cm^2. Garden et al[5] made similar observations; they found HPV DNA in the plume of smoke regardless of power densities or use of the pulse or continuous mode.

When the CO_2 laser is used to treat genital HPV infections, the generated plume of smoke may become contaminated by HPV DNA. The rate of viral dispersion appears to be relatively low by ViraPap, for only one of the five pre-filters (20%) used for treating 65 patients with histologically unequivocal anogenital condylomata and cervical intraepithelial neoplasia was HPV DNA-positive. Whether a different HPV detection technique than the one used in the present study would yield greater HPV DNA positivity remains to be determined. In our opinion, if well-functioning laser smoke evacuator and filter systems are used and if the distal end of the vacuum tube is placed within 1 cm over the lased treatment field, contamination of the operator by laser-dispersed HPV DNA should be an unlikely event. Long-term follow-up studies on a large number of physicians who routinely perform laser treatment for genital HPV infections are needed to provide insight into their relative risk of developing extragenital HPV infections.

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