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Polymerase Chain Reaction for Monitoring Human Papillomavirus Contamination of Medical Personnel during Treatment of Genital Warts with CO_2 Laser and Electrocoagulation

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Genital warts and intraepithelial neoplasia caused by infection with human papillomavirus are usually treated with CO_2 laser or electrocoagulation. In this study, contamination of personnel and operating theatres with human papillomavirus DNA during treatment sessions was investigated. Samples were taken from the nostrils, nasolabial folds and conjunctiva of the operating physician before and after operating sessions and from Petri dishes left open in the operating theatres. Human papillomavirus DNA was demonstrated by the polymerase chain reaction technique. The results show that there is a risk of contamination of the operator by human papillomavirus DNA, detectable with the polymerase chain reaction technique, during both CO_2 laser and electrocoagulation treatment. ***Key words: nasolabial fold; nostrils; conjunctiva; operators; operating theatres; aerosol; protective equipment.***

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In gynaecological practice genital lesions containing human papillomavirus (HPV) are mainly treated with CO_2 laser, whereas in venereological practice electrocoagulation is still the most widely used technique. Both these techniques generate considerable amounts of aerosols containing more or less degraded fragments of wart tissue, thereby posing a definite risk to the operating physician of being contaminated by potentially infectious HPV. Thus, HPV DNA has been detected with dot-blot analysis in the vapour of warts treated with CO_2 laser and electrocoagulation (4). Although a filter hybridization technique failed to detect HPV-DNA from the surgeon before or after CO_2 laser treatment of HPV-DNA-positive lesions (1), concern has been voiced that even small amounts of HPV may constitute a risk to the operating physician. More sensitive techniques are therefore required to determine the degree of viral exposure of the personnel.

Here, we have examined with the polymerase chain reaction (PCR) technique whether CO_2 laser or electrocoagulation treatment of HPV-induced genital lesions is associated with viral contamination of the operating personnel and the operating theatre. Our results show that there is a risk of contamination of the operator by PCR-detectable HPV DNA during both CO_2 laser and electrocoagulation treatment.

MATERIAL AND METHODS

Treatment of HPV lesions

No specific study conditions regarding laser and electrocoagulation equipment were established. The study was conducted under the conditions of our routine clinical work. Patients were not selected specifically for this study but came from our general genital viral warts population. Four to 8 patients were treated per session. The specimens were taken during the winter of 1992–1993.

Electrocoagulation was performed with a Martin-Elektrotom 30 (Martin, Tuttlingen, Germany). The coagulation dose was 15–21W. The resulting char was removed with a cotton swab, and the electrocoagulation was repeated until the lesion was removed.

A Sharplan 1041 CO_2 laser (Laser Industries Ltd, Tel Aviv, Israel) linked to a colposcope (focal length 300 mm) was used either in a focused or defocused mode. Power settings were 20 W with continuous or pulsed emission when treating warts in the vulva, and 40 W when treating dysplasia and/or warts on the cervix uteri. The smoke evacuator was adjusted to draw the vapour away from the laser target.

Collection of samples

Samples were taken from the nasolabial fold and from the conjunctiva with a cotton pin and from the nostril with a cytobrush before and after the CO_2-laser and electrocoagulation treatment session. The samples were incubated in phosphate-buffered saline. During each CO_2 laser session, all personnel wore standard surgical masks (Lazer Surgical Mask, Tecnol, Fort Worth, Texas, USA) and protective plastic goggles. During electrocoagulation sessions, samples were taken both with and without the surgeon wearing a surgical mask.

For detection of HPV-DNA in the operating theatres, 50 mm Petri dishes were left open during the operating sessions, as indicated in the text.

PCR technique

Amplification and typing of HPV DNA sequences was performed as described by Manos et al. (2), and modified by Voog et al. (5). The consensus MY11 and MY09 primers promote amplification of an approximately 450 bp product from at least 25 distinct genital HPVs. Recombinant plasmid DNA containing the entire HPV 6, 11, 16, 18 and 31 genome, respectively, was used as positive controls. Samples negative for HPV DNA were also amplified with β-globin primers to exclude false negative results (3).

Oligonucleotides MY12, MY13, MY14, WD74 and MY16 served as type-specific probes for the consensus PCR products of HPV types 6, 11, 16, 18 and 33, respectively. Each of the specific probes corresponded to a region unique for the particular type of HPV. Aliquots of the PCR products were denatured and applied to blot membranes by standard procedures. The membranes were hybridized with each of the type-specific biotinylated probes. ECL detection and autoradiography were performed according to standard protocols.

RESULTS

The sensitivity of the PCR was determined by performing a PCR on diluted HPV plasmid DNA. One fg of cloned HPV

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Fig. 1. Visualisation of positive HPV polymerase chain reaction after agarose gel elctrophoresis and ethidium bromide staining. The *arrows* indicate positions of DNA fragments of 1033, 653, 517, 453, 394 and 298 basepairs, respectively. (*A*) DNA markers; (*B*) and (*C*) a clinical specimen from the study run in duplicate, showing a weakly positive PCR reaction; (*D*) and (*G*) blank (water) PCR reactions, run in parallel with the clinical specimens, physically separating the specimens in the reaction plate; (*H*) and (*J*) a strongly positive clinical specimen; (*K*), (*L*), and (*M*) positive controls, using 100, 10, and 1 fg, respectively, of HPV type 18 DNA.

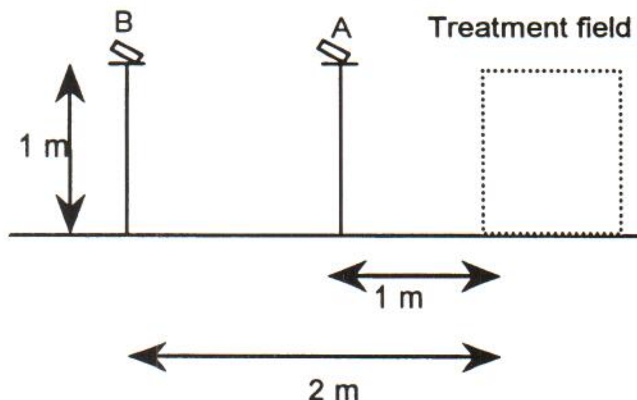

Fig. 2. Diagram showing the set-up of Petri dishes for isolation of aerosol HPV DNA. In order to trap material from laterally streaming air from the treatment field more easily, the Petri dish was tilted as indicated in the figure.

genome could be detected, which corresponds to about 100 copies of the HPV genome. Examples of positive and negative results are shown in Fig. 1. The control runs with β-globin primers revealed no false negative results due to PCR incompatibility of the analysed specimens. Specimens were collected from the operating personnel before and after each treatment session, as indicated in Table I.

For personnel performing diathermic treatment, HPV DNA was found in 32% of the post-session specimens collected from the nasolabial folds, whereas only 16% of the corresponding nostril cytobrushes were positive. The staff members being positive for HPV DNA in the nostrils also had PCR-positive nasolabial fold specimens. All conjunctival swabs were negative. All pre-session samples were negative for HPV DNA, except two samples taken before two consecutive sessions from the nasolabial fold of one and the same staff member, who was also in the group having PCR-positive post-session nasolabial fold specimens. In order for us to see whether this pre-session PCR positivity was caused by HPV contamination irrelevant to this study, e.g exposure to virus shed by family members, off-duty specimens were taken when the operator entered the hospital building on three occasions separated by at least 18 h. None of these specimens became positive for HPV DNA (data not shown), suggesting that the pre-operative positivity was the result of some work-related environmental factor. The significantly higher rate of PCR positivity post-operatively indicated that direct contamination of the operator by HPV or its DNA from the vapour plume generated by electrocoagulation is a common phenomenon, unless precautions are taken.

Table I. *Total evaluation of PCR analysis of medical personnel*

Treatment	Specimen from treating physician	No HPV-positive/Total No	
		Before treatment session	After treatment session
Electrocoagulation	Nasolabial fold	2/19	6/19
	Nostril	0/19	3/19
	Conjunctiva	0/13	0/13
CO_2 laser	Nasolabial fold	2/11	3/11
	Nostril	3/11	3/11
	Conjunctiva	0/6	0/6

DISCUSSION

The results from the unit performing laser therapy indicated a much higher degree of pre-session contamination (Table I). Thus, both pre-session nostril and nose-wing specimens were positive in altogether 5 cases, and only one additional positive result was recorded in the post-session specimens. These results indicate that the operating staff at the gynaecology department were subjected to an environmental exposure to HPV DNA even before or between the operation sessions. The finding that only one additional HPV-positive operator was discovered by analysing the post-operative specimens suggested that contamination during the treatment session was a smaller problem for laser operators than for electrocoagulation operators, the consequence of a consistent use of surgical masks in combination with protective goggles.

To determine the probability of the pre-session contamination being due to HPV contained by persistent aerosols generated by previous sessions, we tried to detect surface deposits of HPV DNA by using opened Petri dishes. The dishes were exposed for different periods of time and at two different distances from the treatment field, as indicated in Fig. 2. The results from PCR determinations of material extracted from the Petri dishes are given in Table II. The most striking difference between the

Table II. *Distribution of HPV in the treatment units*

	HPV occurrence (number of positives/total number of samples)	
Treatment	A (1 m)[1]	B (2 m)
CO_2 laser	ND[2]	2/5
Electrocoagulation	0/5	0/5

[1]Sample collection position as indicated in Fig. 2.
[2]ND= not done.

results obtained from the two departments investigated was that whereas HPV DNA was demonstrated on surfaces situated as far as 2 metres from the treatment field in the gynaecology department, no HPV DNA at all was detected in the Petri dishes exposed in the electrocoagulation unit. Thus, the high level of pre-operative contamination in the gynaecology department correlated well with the long-distance spread of aerosol-bound HPV DNA demonstrated by the exposed Petri dishes. These data would suggest that the droplets and fragments from the electrocoagulation treatment are physically distinct and much less prone to be transported than the aerosol generated by laser treatment.

We addressed this problem further by examining the post-operative routines at the gynaecology department and found that the procedures for handling personal protective equipment could result in the deposition of small but significant amounts of HPV-contaminated material on non-disposable protective equipment. The investigation prompted revision of these routines, including a chemical decontamination step. A preliminary evaluation of the outcome of this change is encouraging, but further studies are needed before definite conclusions can be drawn. The demonstration of HPV DNA on surfaces up to 2 metres from the treatment area suggested, but did not prove, that aerosol deposits were the cause of the pre-session contaminations.

In conclusion, our result suggest that there is a definite risk of HPV DNA contamination of the operator during both types of treatment. The frequency of pre-session contamination is higher for laser treatment than for electrocoagulation treatment, probably due to less persistent HPV DNA-containing aerosols during the latter type of treatment. Our data allow no conclusions as to whether the PCR-positive results reflect the presence of infectious particles, withstanding the thermal stress of the treatment process, or non-infectious DNA fragments. We typed positive amplificates found on the nasolabial fold prior to treatment session, as described in Material and Methods, but none of the amplificates were derived from HPV types 6, 11, 16 or 31, covered by our type-specific probes. We recommend the use of approved face masks, evacuation of air in the vicinity of the treatment field whether laser or electrocoagulation is practised, and thorough decontamination of the personal protective equipment after each session. These precautions should be sufficient to eliminate potential risks of contamination by HPV occasioned by both electrocoagulation and laser treatment of genital warts. However, further studies would be needed to evaluate not only the degree of HPV contamination of different parts of surgical equipment but also the risk of inter-patient contamination.

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