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INVESTIGATIVE REPORT

Low Prevalence of Oral and Nasal Human Papillomavirus in Employees Performing CO₂-laser Evaporation of Genital Warts or Loop Electrode Excision Procedure of Cervical Dysplasia*

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Risk of human papillomavirus (HPV) transmission during laser vaporisation of genital warts or loop electrode excision procedure is controversial. An oral rinse, a nasal swabs, history of HPV-related diseases and data on HPV exposure were collected from 287 employees at departments of dermato-venereology and gynaecology in Denmark. A mucosal HPV type was found among 5.8% of employees with experience of laser treatment of genital warts as compared to 1.7% of those with no experience ($p=0.12$). HPV prevalence was not higher in employees participating in electrosurgical treatment or cryotherapy of genital warts, or loop electrode excision procedure compared with those who did not. HPV 6 or 11 were not detected in any samples. Hand warts after the age of 24 years was more common among dermatology than among non-dermatology personnel (18% vs. 8.0%, $p=0.03$). Mucosal HPV types are infrequent in the oral and nasal cavity of health care personnel, however, employees at departments of dermato-venereology are at risk of acquiring hand warts. *Key words:* human papillomavirus; HPV; plume; LEEP; carbon dioxide laser; genital warts; condyloma acuminatum; hand warts.

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Genital warts are caused by human papillomavirus (HPV), which is the most common sexually transmitted

infection (STI) (1–3). At least 40 HPV types can infect the genital mucosa (4) and they are classified as oncogenic (high-risk) or non-oncogenic (low-risk) HPV types, on the basis of their association with malignant genital lesions (5). The majority of genital warts is caused by the low-risk HPV types 6 or 11 (6, 7). Molecular and epidemiological studies have demonstrated the role of genital high-risk HPV, in the aetiology of oro-pharyngeal cancers (8, 9) and HPV types 6 and 11 is the cause of both juvenile- and adult-onset laryngeal papillomas (10).

Genital HPV infections mainly spread by direct genital contact, especially in areas of epidermal barrier erosion or friction (11). Other routes of HPV transmission are incompletely understood. Oral HPV infection may be related to orogenital or oral-to-oral spread in adults (12, 13).

Occupational HPV transmission from patient to medical personnel during carbon dioxide (CO₂) laser vaporisation of genital warts or loop electrode excision procedure (LEEP) of cervical dysplasia is controversial. Studies have shown that HPV DNA is present in the plume generated during laser vaporisation of verrucae, laryngeal papillomas and genital warts (14–16). HPV 16 is the most frequently isolated HPV type from plume samples taken during LEEP (17). Two cases of laryngeal papillomatosis among operating personnel who used laser vaporisation to treat genital warts have been described (18, 19). This has resulted in increased use of personal protective equipment and smoke evacuators in Denmark during the last decade; which may be sufficient to protect medical personnel from acquiring HPV infection (20, 21).

To investigate the prevalence of mucosal HPV types in the nasal and oral cavity and history of HPV-related diseases in relation to work exposures, we collected oral rinses, nasal swabs and information on HPV related diseases from medical personnel at departments of gynaecology and dermato-venereology in Denmark. Results from the same cohort have been published in a previous paper (22). That paper focus on the presence

*Part of the data has been presented at the 28th International Papillomavirus Conference (Puerto Rico 2012), The 6th International Conference of HPV, Polyomavirus and Ultraviolet Radiation in Skin Cancer (Berlin 2012) and the 72nd annual meeting of the American Academy of Dermatology (Denver 2014).

of HPV types from the *Betapapillomavirus* and *Gamma-papillomavirus* genera in the nasal mucosa and does not contain results regarding the work-related exposition to HPV, work place or history of HPV-related diseases.

METHODS

Participants

Medical personnel employed at departments of gynaecology and dermato-venereology was invited to participate. The participating departments of dermato-venereology were located at Copenhagen University Hospital Gentofte and Bispebjerg, Odense University Hospital, and Aarhus University Hospital. The participating departments of gynaecology were located at Copenhagen University Hospital Hvidovre, Herlev and Hilleroed, Odense University Hospital, and Aarhus University Hospital. The total number of medical personnel at the participating departments of dermatology is approximately 290, and approximately 1,320 medical personnel are working at the participating departments of gynaecology. The number of employees with experience of treating patients with HPV-related diseases is not known. Additionally, employees at a department at the medical faculty at Copenhagen University were invited to participate. The study was performed between 2010 and 2011. Participants had to be at least 18 years of age and should not have treated patients with HPV-related diseases for 24 h before sampling. The Scientific Ethical Committee of the Capital Region approved sample collection based on written informed consent (H-D-2010-077). All patients were offered access to their study results and subsequent counselling if needed.

After sampling the participants answered a questionnaire concerning demographic data, previous and current work-related HPV exposure, and history of HPV-related diseases.

Sample collection

An oral rinse was obtained by means of a ≈ 30 s oral rinse and gargle with 7 ml isotonic saline collected in a 10 ml tube. Nasal samples were collected using a swab applicator with a nylon fibre flocked tip moistened in isotonic saline. The applicator was placed ≈ 1 cm inside the nostril, and with moderate pressure against the nasal septum rotated 3 times in each nostril. Subsequently the applicator was placed in a cryo tube containing 1.5 ml isotonic saline solution.

Analysis

After purification and amplification a Luminex-based HPV genotyping was used to identify HPV types as previously described (7, 23). The technique allows the detection of at least 39 HPV genotypes of which 15 are high-risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82; and 5 probable high-risk types genotypes: 26, 53, 66, 67 and 69, and 19 low-risk HPV genotypes: 6, 11, 30, 40, 42, 43, 54, 61, 62, 70, 74, 81, 83, 86, 87, 89, 90, 91 and 114. The Luminex assay also included 2 "universal" HPV probes and samples positive only for the "universal" probe were typed by DNA-sequencing. β -globin real-time PCR was included as a separate test of sample adequacy for PCR (7). Only participants with β -globin positive samples were included in the analysis.

Statistics

Qualitative variables were given as number (percentage) and were studied using Fisher's exact test. Quantitative data were expressed as mean (\pm standard deviation) or median (range) as appropriate.

Data analysis was conducted using SAS statistical software (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). p -values < 0.05 were considered to be statistically significant.

RESULTS

A total of 314 persons participated. Two did not fill out the questionnaire and were excluded and 25 participants from the medical faculty were excluded because they had not worked at a department of dermato-venereology or gynaecology. Characteristics of the 287 persons are given in Table SI¹. As shown in Table SII¹ there was no difference in the history of HPV-related diseases, warts since the age of 25 years or warts at inclusion between physicians and non-physicians. Participation in CO₂ laser treatment of HPV-related diseases did not influence the reported history of HPV-related diseases. A significantly higher number of employees at a department of dermato-venereology reported having had an HPV related disease in their lifetime (OR 1.9; 95%CI 1.1-3.2). This was mainly due to significantly employees reporting hand warts after the age of 24 years (OR 2.2; 95%CI 1.1-4.5). Three persons, all of whom were employees at a department of dermato-venereology reported nasal or oro-pharyngeal warts after the age of 24 years.

Due to a β -globin negative oral rinse or a nasal swab 16 persons were not included in the analysis of oral or nasal HPV. HPV was isolated from 13 persons, types and sites are given in Table SIII¹. The HPV type isolated from 2 participants was not regarded as a mucosal HPV type (HPV 10 and a putative subtype of a *Gammapapillomavirus* HPV isolate) and thus not included in the comparative analysis.

A mucosal HPV type was found among 5.8% of employees with experience of laser treatment of genital warts as compared to 1.7% of those with no experience ($p=0.12$) (Table I). HPV 6 or 11 were not detected in any of the samples, while HPV 16 or 18 were found in 4.

Employees participating in CO₂ laser treatment of genital warts, had been doing the procedure for a median of 5 years (range 0-25). Laser personnel that treated patients with genital warts for at least 5 years had a significantly higher prevalence of mucosal HPV types in the nasal or oral cavity than employees that used CO₂ laser for less than 5 years or never (OR 6.7 (95% CI 1.7-26.0; $p=0.004$)). The median age of employees with ≥ 5 years experience of CO₂ laser treatment was 49 years (range 36-68), while the median age of personnel with less than 5 years or no experience of laser treatment was 40 years (range 22-64). Persons performing cryotherapy or LEEP did not have a higher prevalence of mucosal HPV compared with those who did not. The prevalence of mucosal HPV was not significantly higher

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1912>

Table I. Mucosal human papillomavirus type in relation to exposure

Work exposure and position	HPV+ <i>n</i> (%)	HPV− <i>n</i>	<i>p</i> ^a
Treating human papillomavirus (HPV) related diseases			
Yes	11 (4.9)	213	0.22
No	0 (0.0)	47	
CO ₂ -laser treatment of genital warts			
Yes	9 (5.8)	147	0.12
No	2 (1.7)	113	
Cryotherapy of genital warts			
Yes	4 (4.1)	93	1.00
No	7 (4.0)	167	
Electrosurgical treatment of genital warts			
Yes	6 (6.5)	87	0.20
No	5 (2.8)	173	
Loop electrosurgical excision procedure			
Yes	5 (4.7)	101	0.76
No	6 (4.6)	159	
Occupation			
Physician	8 (5.3)	143	0.36
Non-physician	3 (2.5)	117	
Work place			
Dermato-venerology	6 (6.3)	90	0.18
Gynaecology	5 (2.9)	170	

^aFisher's exact test.

in employees participating in electrosurgical treatment of genital warts (Table I).

All persons currently involved in CO₂ laser treatment, reported taking some protective measures against HPV infection. All except 2 (93%) used examination gloves, 88% used smoke evacuators and 79% laser plume masks. The majority (64%) of medical personnel who stopped using CO₂ laser in the treatment of genital warts ≥ 5 years before the study was conducted took no protective measures against HPV infection or wore only examination gloves.

DISCUSSION

In our study we found that participating in cryotherapy, CO₂ laser or electrosurgical evaporation of genital warts or LEEP of cervical dysplasia did not significantly increase the prevalence of nasal or oral HPV.

In a recent study of medical personnel treating laryngeal papillomas and urethral warts using CO₂ laser it was found that after treatment of urethral warts, HPV DNA corresponding to patient tissue specimens was present in all samples obtained from the gloves of the surgeons (21). Oral mucosa samples from all 18 different employees tested HPV negative, as did the surgical mask specimens. Similar results have been found by others (20, 24). Thus, it seems that wearing a laser plume mask or even a surgical mask protects from upper airway HPV infection. Or it may be that regardless of protective measures the risk of HPV infection by inhalation of laser plume is very small. Bellina et al. (24) showed that only a few morphologically intact cells are present in the plume collected during CO₂ laser treatment of genital warts and

incubation of the cellular debris resulted in no metabolic activity, replication or transcription of HPV. On the other hand, Garden et al. (25) treated bovine papillomavirus (BPV)-induced cutaneous fibropapillomas on calves with CO₂ laser. The laser plume was collected and then re-inoculated onto the skin of other calves. Fibropapillomas developed at laser plume-inoculated sites.

We did not detect HPV 6 or 11, which is by far most frequently encountered types in genital warts, in a single employee (6, 7). However, we did find an elevated prevalence of nasal or oral HPV in employees that had participated in CO₂ laser for at least 5 years. This may simply be due to an increasing number of nasopharyngeal HPV infections with age, which have previously been shown (13, 26). Only 13 (4.4%) employees had a mucosal HPV positive sample. In another study collected oral mucosa specimens from 18 health care professionals all tested HPV negative (21). The reported prevalence of HPV in oral rinse samples in the United States among men and women aged 14–69 years is 6.9% (13). In that study the prevalence of HPV 16 and 18 were 1% and 0.25%, respectively, which is approximately the same as detected in our study. A similarly low prevalence of high-risk HPV types (0.63%) was found in mouthwash from Swedish healthy controls (27). However, among normal oral cavity scrapings taken with a brush Rautava et al. (28), reported HPV DNA prevalence of 17% in Finnish pregnant women.

We found that employees at a department of dermatovenerology have a greater prevalence of hand warts and possibly nasal or oral warts, than employees at departments of gynaecology. In a study based on a self-administered questionnaire, 4 out of 570 U.S. CO₂ laser surgeons reported a history with nasopharyngeal warts. This small number was significantly higher than the number observed in matched control subjects (29). However, when warts were grouped together without specification of anatomic site the reported prevalence of warts among the laser surgeons was not significantly different from the prevalence in the control group (29). Lobraico et al. (30) conducted a survey by a questionnaire sent to laser users, and discovered a significantly higher prevalence of acquired hand warts among the dermatologists compared to other specialties. However, genital warts and warts of the skin are induced by an entirely different set of HPV.

Our study has some limitations. Detection of HPV is dependent on sampling procedure and assay sensitivity and specificity. In the assay and sampling procedures we used may not have been able to detect low productive infections e.g. in the tonsillar crypts. When asked in hindsight every specialised health care professional may have a stronger recall of diseases, which belong to their own area of experience than the ones that do not. Even though this study is by far the largest on the prevalence of nasopharyngeal HPV in medical personnel it might still be too small to detect important

infrequent associations between nasopharyngeal HPV prevalence and work exposure. Most HPV infections are transient and thus the prevalence deduced by point testing with swab and rinses are not able to measure the transmission rate over a period of years.

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Conflicts of interest. KK has received fees as a speaker and obtained research grants from Sanofi Pasteur MSD. CS has obtained research grants and received fees as a speaker and for Sanofi Pasteur MSD. KGM is the former managing director at Sanofi Pasteur MSD Denmark. The study was supported by a research grant from Sanofi Pasteur MSD, Denmark.

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