



Is surgical plume developing during routine LEEPs contaminated with high-risk HPV? A pilot series of experiments

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Abstract

Introduction Growing evidence shows a causal role of high-risk human papillomavirus (HPV) infections in the development of head and neck cancer. A recent case report shows two patients suffering from tonsillar cancer without any risk factors apart from their work as gynecologists doing laser ablations and loop electrosurgical excision procedures (LEEP). The aim of the present investigation is to evaluate whether surgical plume resulting from routine LEEPs of HSIL of the cervix uteri might be contaminated with the DNA of high-risk HPV.

Materials and methods The prospective pilot study is done at the Department of Gynecology and Obstetrics of the University of Lübeck, Germany. The primary outcome was defined as HPV subtype in resected cone and in surgical plume resulting from LEEPs of HSIL of the cervix uteri. Plume resulting from LEEPs was analyzed using a Whatman FTA Elute Indicating Card which was placed in the tube of an exhaust suction device used to remove the resulting aerosols. For detection of HPV and analysis of its subtype, the novel EUROArray HPV test was performed. Resected cones of LEEPs were evaluated separately for HPV subtypes.

Results Four samples of surgical plume resulting from routine LEEPs indicated contamination with high-risk HPV and showed the same HPV subtype as identified in the resected cones.

Conclusion Surgical plume resulting from routine LEEPs for HSIL of the cervix uteri has the risk of contamination with high-risk HPV. Further investigations of infectiousness of surgical plume are necessary for evaluation of potential hazards to involved healthcare professionals.

Keywords Human papilloma virus · Plume · Loop electrosurgical excision · High-grade squamous intraepithelial lesion · Cervical dysplasia

Introduction

High-risk HPV is known to be of growing importance in the development of head and neck cancer [1, 2]. A recent case report shows two gynecologists suffering from human papillomavirus (HPV) 16 positive tonsillar cancer without any risk factors apart from doing laser ablations and loop electrosurgical excision procedures (LEEP) for many years [3]. LEEPs of the uterine cervix are the most common method for excision of high-grade squamous intraepithelial lesions (HSIL) which originate on the basis of chronic cervical infections with high-risk HPV [4, 5]. In the US, about half a million LEEPs are done per year [6]. So far, investigations of HPV contamination of plume resulting from surgeries (often referred to as “surgical plume”) focused mainly on oropharyngeal operations (e.g., of papillomas) or laser ablations of condylomas. For these operations, contamination

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with low-risk HPV has been shown. But its potential for infections has remained contradictory [7–10]. For protection of the involved healthcare professionals, the use of efficient smoke evacuation systems in combination with FFP2 respiratory masks is mandatory for laser ablations of condylomas in Germany. This is in contrast to guidelines for LEEPs which do not require specific respiratory masks for involved healthcare professionals. It was assumed that aerosols resulting from cutting with high temperatures might be less likely contaminated than surgical plume resulting from resections with low temperatures [8, 11]. Therefore, little risk for contamination of surgical plume from LEEPs was presumed due to the utilization of electrical cutting.

The aim of the present investigation is to investigate whether it is feasible to detect contamination with high-risk HPV of surgical plume resulting from routine LEEPs of HSIL of the cervix uteri.

Materials and methods

Experimental setting

The analysis of surgical plume resulting from routine LEEPs of HSIL of the cervix uteri was performed at the Department of Obstetrics and Gynecology at the University hospital of Luebeck, Germany. Institutional review board approval was granted for non-invasive investigation of dysplasia (Ethical Review Board of the University of Luebeck, reference number AZ-12232). All the patients consented in investigation of the obtained data. Patients received LEEPs because of the cytological and histological evidence for HSIL at the transformation zone of the cervix uteri. Prior to the operation, patients received colposcopy, Pap smear testing and/or histological biopsies of the dysplastic lesions. All the operated patients had cytological and/or histological evidence for high-grade intraepithelial squamous lesions and were, therefore, treated by LEEP for further diagnostic and therapeutic indications. The histological analysis was performed by the Institute of Pathology of the University of Luebeck. Resected cones

were embedded in formalin and later stained by haematoxylin–eosin staining for microscopic evaluation. For the comparison of HPV subtypes, resected cones were tested separately using the HPV EUROArray (EUROIMMUN, Luebeck, Germany) for HPV subtype identification [12].

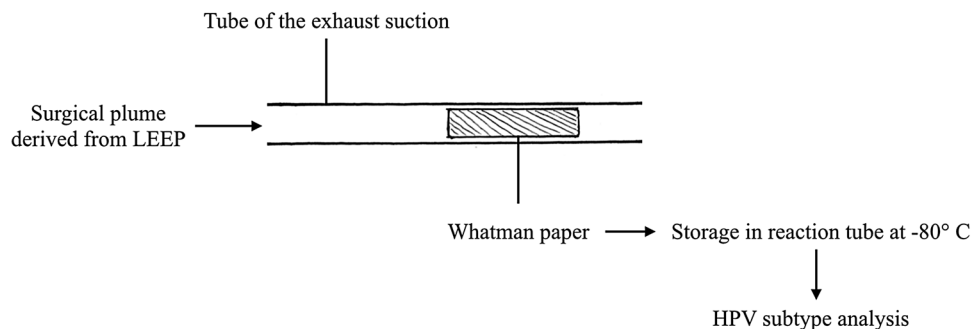
Sample collection

LEEPs are performed routinely at the Department of Obstetrics and Gynecology at the University Hospital of Luebeck. Before the LEEP, a speculum was used to expose the dysplastic areal of the cervix. For staining of dysplastic areas, Lugol's solution was used. The size of the chosen wire loops reflected the affected area of the cervix uteri. A smoke evacuation system was used to collect the developing plume. It was held close to the loop, but was not in direct contact with the cervix uteri during resection of the cone. Thus, it was ensured that only plume and no other tissue particles were collected by the smoke evacuation system.

During the resection of the cone, the emerging smoke was aspirated with a vacuum pump. In the tube of the exhaust suction device, a two square centimeter piece of the indicating field of a Whatman FTA Elute Indicating Card was placed and humidified with 200 μL dH_2O , so that parts of the smoke may accumulate in the Whatman paper. A draft of the experimental setting is shown by Fig. 1. FTA Cards are impregnated with a chemical formula that lyses the cell membranes and denatures proteins on contact. Nucleic acids are physically entrapped, immobilized and stabilized.

After the LEEP, the Whatman paper was removed from the tube with a sterile forceps, placed in a 1.5 ml reaction tube and stored at -80°C . For the elution of the nucleic acids, five samples were punched out of the paper with a sterile 3 mm punch and placed into a sterile microcentrifuge tube. To rinse the punches, 500 μL of dH_2O was added and the punches were vortexed three times for 5 s. After removing the water with a sterile pipette, 50 μL sterile dH_2O was added and the samples were incubated for 30 min at 95°C to elute the DNA from the paper.

Fig. 1 Draft of the experimental setting



Analysis of HPV subtypes of resected cones using the EUROArray

Additionally, the pathologic formalin-fixed, paraffin-embedded (FFPE) tissue of the resected cones of the patients were analyzed for HPV subtypes. Four 10 µm slices of the FFPE tissue were placed in a 1.5 ml reaction tube, overlaid with 1 ml xylene and rigorously vortexed for 10 s. After 2 min (min) centrifugation with 20,000×g, the supernatant was discarded. The tissue was washed with 1 ml 96% (v/v) ethanol in a short vortexing step. After 2 min centrifugation with 20,000×g, the supernatant was discarded. The cell pellet was air dried at 37 °C for 10–20 min and resuspended in 180 µl puffer ATL. Afterwards, the DNA was prepared conform to the instructions of the QIAamp DNA Mini Kit (QIAGEN).

A 5 µl aliquot of DNA of the tissue and smoke samples were tested with the EUROArray HPV test system according to the manufacturer's instructions. The test is designed for molecular diagnostic in vitro detection and typing of all the 30 relevant anogenital high-risk and low-risk papilloma viruses subtypes (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 70, 72, 73, 81, 82, 89 (CP6108)) from the DNA preparations [12].

Results

Patient population

Overall, in a pilot study design, 24 patient samples were obtained in various settings. In the above presented setting (see “Materials and methods”), four plume samples were indicated positive for high-risk HPV by the HPV EUROArray. The age of these patients on date of LEEP ranged from 29 to 65 years with a mean of 41 years and a standard deviation (SD) of ± 16.57 years. None of the patients smoked, two were married, none received HPV vaccination previous to LEEP and all had cytological evidence for HSIL. The histological results of cones resected in LEEPs showed HSIL for three patients, one resected cone lacked evidence for dysplasia.

HPV subtype analysis

Analysis of HPV subtypes of surgical plume samples detected in one sample HPV 16, one showed HPV 39 and two indicated HPV 53. Testing of it in the LEEP resected cones confirmed for each sample the same HPV subtype as was detected in the plume sample received by LEEP. Table 1 shows the detected HPV subtypes.

Table 1 Detected HPV subtypes

	Plume sample of LEEP	Tissue sample of LEEP
Patient nr. 1	16	16
Patient nr. 2	39	39
Patient nr. 3	53	53
Patient nr. 4	53	53

Discussion

The present pilot study shows that it is feasible to detect contamination with high-risk HPV of surgical plume resulting from routine LEEPs of HSIL of the cervix uteri in 4 out of 24 cases. Comparison of HPV subtypes of the resected tissue confirms the same HPV subtype as was detected in the plume sample received during LEEP. These results are sufficient to indicate a risk of high-risk HPV contamination of surgical plume of routine LEEPs despite the low number of cases in this pilot study design. Additionally, a previous investigation from 1994 supports this finding of high-risk HPV contamination of surgical plume [13].

However, the capability for infectiousness of these HPV particles remains unclear. For low-risk HPV subtypes obtained from surgical plume of operations of warts, there is experimental evidence for potential infectiousness [14]. But for surgical plume contaminated with high-risk HPV, further investigations are necessary to evaluate the potential for infections (e.g., of healthcare professionals).

HPV is classified in the European Union as a risk group two virus which defines it as an existing but moderate hazard to healthcare professionals [15, 16]. It is considered most important for protection to avoid inhalation of HPV aerosols by the use of an efficient suction device [8].

Furthermore, respiratory masks are known to offer efficient protection from the viruses [8, 14]. Prospectively, it might be reasonable to utilize the same standard as it is mandatory for laser ablations (e.g., use of FFP2 masks which come with higher protection against air contamination), until there is definite knowledge about the potential infectiousness of high-risk HPV particles in surgical plume. Additionally, HPV vaccination against a broad spectrum of subtypes (e.g., by Gardasil 9) could be a useful approach for further protection of healthcare professionals involved in LEEPs.

In summary, the present investigation shows surgical plume resulting from routine LEEPs has the risk of contamination with high-risk HPV. Further investigations of infectiousness are necessary for evaluation of potential hazards to the involved healthcare professionals.

Compliance with ethical standards

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Conflict of interest There are no conflicts of interest for any of the authors.

Ethical standards All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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