Oral Oncology 50 (2014) 27–31

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 11 July 2013
Accepted 8 October 2013
Available online 26 October 2013

Keywords:
Oropharyngeal carcinoma
HPV
Surgery
Survival
Risk factors
Sexually transmitted infection (STI)
Oral-genital infection

SUMMARY

Background: Oncogenic human papillomaviruses (HPV) are known to be associated with carcinomas of the uterine cervix. Furthermore, current studies have shown that HPV-infection is also associated with a subtype of oropharyngeal cancers. In general, a sexual transmission of the viruses has been shown by numerous studies in the genital lesions. However, there are unknown factors regarding the prevalence and transmission of HPV in the oropharynx.
The aim of this study was to evaluate HPV prevalence in the oropharynx in female participants with and without genital HPV infection. In addition, we analyzed risk factors for an oropharyngeal colonization with HPV in their sexual partners, too.
Methods: 129 Female participants were tested for presence of HPV-DNA by oral lavage, brush cytology of the tonsils and of the cervix. In addition, 15 male partners of these patients were included in the study. HPV-DNA was detected by PCR (polymerase chain reaction) amplification. For HPV-genotyping, PCR products were hybridized with type-specific digoxigenin-labeled oligonucleotide probes and discriminated into 14 high risk (HR) and 6 low risk (LR)-HPV types. The 129 female and 15 male participants were interviewed by a standardized questionnaire for socioeconomic details, drinking, smoking and sexual behaviours.
Results: 59 (45.7%) Female participants were negative for a genital HPV-infection. Of these women, 3 (5.1%) showed a positive HPV-PCR result (HR and LR) in the oropharynx. 70 (54.3%) Female participants were positive for a genital HPV infection. In this group, 4 (5.7%) had a positive HPV-detection (HR and LR) in the oral cavity and oropharynx. Female participants with cervical HPV-infection had no higher risk for HPV-detection in the oropharynx (not significant). The analysis of sexual risk factors revealed no specific risk factor for an oral HPV-infection.
Conclusion: A correlation between cervical and oral colonization by HPV could not be demonstrated in our small cohort. Our limited data suggest that sexual transmission of HPV from the cervix uteri to the oropharynx is a rare and unlikely event.

Introduction

Genital sexual intercourse was documented as one of the most important risk factors for human papillomavirus (HPV) infection in carcinomas of the uterine cervix. According to current studies, male partners seem to play an important role in transmission of HPV to their female partners [1]. In up to 56% of heterosexual partners of genital HPV-positive women, a concordance of at least one viral subgroup of HPV was detected in samples of the partner's penis [1]. A higher infection rate could be detected from the cervix to the penis than vice versa [2].

HPV can be detected in almost all carcinomas of the uterine cervix. Particularly the high-risk HPV types 16 and 18 cause a malignant transformation of the cervix squamous cell tissue and

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http://dx.doi.org/10.1016/j.oraloncology.2013.10.009

may lead to cancer [3]. It could be shown that women with HPV-positive cervical smears have a higher morbidity rate for cervical cancer than HPV-negative women [3]. However, HPV is regarded not only as a significant risk factor for carcinoma of the uterine cervix and anal carcinoma but also for squamous cell carcinoma of the head and neck (SCC) and especially oropharyngeal squamous cell carcinoma (OSCC) [4–8]. Thus, for a few years it has been known that high-risk-HPV can cause a malignant transformation of epithelial cells in the oropharynx accounting for a new risk factor in the development of an OSCC in addition to the established risk factors tobacco and alcohol consumption [9–11]. Current studies therefore distinguish two groups of head and neck carcinomas, namely HPV-induced and HPV-negative carcinomas [12,13]. Several studies show that the prognosis is superior in HPV-positive carcinomas compared to HPV-negative carcinomas. A reason for this may be the better response to radiotherapy and chemotherapy, even though patients present with a more advanced stage of disease [5,9,13].

At least 30–40 oncogenic HP-Viruses are known today [5], but HPV16 is the most important type found in 84–93% of the HPV-induced OSCC [5,8,14–18]. In Germany about 30–40% of oropharyngeal carcinomas are classified as HPV-induced [9].

However, the detection rates and incidence vary in several studies. This variation depends on different factors including study models and different detection methods used [19]. Especially, not all tumors tested positive are etiologically HPV related [20,21].

This may be due to the different specificity and sensitivity of the used detection methods for presence of HPV, namely in situ-hybridisation [22,23], polymerase chain reaction (PCR) [18,24] and immunohistology of the surrogate marker $p16^{INK4A}$ [23,25]. A large metaanalysis from 2005 analyzed 60 studies based on PCR-analysis [15]. HPV-prevalence was significantly higher in OSCC specimen (35.6% HPV-positive samples in 969 tumors) than specimen of oral (23.5% HPV-positive samples in 2,642 tumors) or laryngeal (24.0% HPV-positive samples in 1435 tumors) squamous cell carcinoma [15].

In the US population 2–10% individuals show positivity for oncogenic HPV in the oral cavity [10,15,26–28]. HPV transmission is thought to be caused by direct contact with a HPV-infected person or indirect by inoculation of virus-positive squamous cells. Due to the specificity of the virus, an infection is only possible via epithelial and squamous tissue [5,8,9]. A micro trauma is a premise for the infection to the basal cells. After an incubation time of a few weeks up to years, high-risk HPV are able to transform cells into immortal cancer cells with specific biological characteristics [5,10,15,17].

As it is well known that sexual behaviours have an impact on the development of cervical carcinoma, it was thought that sexual behaviours might also have an influence on the risk for developing an OSCC [10,29]. The number of vaginal and oral sex partners, as well as sexual intercourse with subjects with seropositivity for HPV16 is discussed to raise the risk of developing an OSCC [10,11,29]. It could be shown that the risk for developing an HPV-associated OSCC in male patients is higher in case of a history of an abnormal Papanicoulaou (PAP) smear classification and a cervical dysplasia of the female partner [10,11].

The aim of this study was to analyze the prevalence of an oral HPV infection in relation to an existing HPV-infection of the cervix uteri. We also aimed to explore the risk factors for oral and genital HPV infection. Furthermore, where available, we examined oral HPV infection in male partners of those subjects with genital HPV infection.

Methods

Between 2005 and 2006, samples from a consecutive clinical cohort of patients were collected. We could obtain samples from 144 participants (129 female participants and 15 male partners) at the Department of Obstetrics and Gynecology, University Hospital of Cologne, Cologne, Germany. HPV positive (54%) and negative (46%) female participants from the dysplasia consultation hour were included in the study. Additionally, 15 male partners of the 129 female participants agreed to participate in this study. All participants provided informed written consent before being included in this study.

The following samples were collected.

Oral lavage (mouthwash sample)

Participants gargled with 5 ml saline for 15 s. The resulting suspension was filled in a tube containing 0.5 μl Merthiolat®.

Smear of the tonsils

Superficial scrapes of the mucosa of the tonsils were carried out with a Cytobrush® Plus GT, by performing 5–10 complete backward and forward brushes at each oral site (right tonsil, left tonsil). Cells from brushes were suspended in tubes containing phosphate-buffered saline (PBS).

Smear of the cervix

Superficial cervical cells were obtained by using a Cytobrush® Plus GT. Cell samples were taken from the portio and the endocervix. The brushes were suspended in tubes containing 4 ml phosphate-buffered saline (PBS).

DNA isolation and HPV typing by PCR

For DNA isolation tissues were processed with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). After collecting the samples they were centrifuged with the cytobrush and the brush was removed. Subsequently the specimens were centrifuged again at 18.000 rcf (Relative centrifugal force) and the supernatant was collected in a new tube. DNA isolation was performed according to the manufacturer's instructions. Total cellular DNA was eluted with 100 μl (oral samples) or 200 μl (uterine cervical samples) of the AE-buffer (Qiagen) and 5 μl were used in each of the PCR analyses.

To test the quantity and quality of the DNA samples and to demonstrate that the samples were free from inhibitory substances, PCR was performed for the beta-Globin gene, resulting in a 268 bp PCO4/GH20 PCR product. [14]. Specimens with negative results were excluded from subsequent analysis [30]. Tests were performed in duplicate.

HPV-sequences were detected by highly sensitive group-specific nested PCR protocols with degenerate primers A5/A10 and A6/A8 for HPV as previously described quote. The sensitivity of the nested PCR was tested by defined HPV16 DNA and was approximately under 10 DNA copies. Five μl of these PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining. For HPV-typing, internal biotinylated A6/A8-PCR products (270 bp) were hybridized with type-specific digoxigenin-labeled oligonucleotide probes (HR, 14 types; LR, 6 types) in an enzyme-immunoassay as described earlier quote.

Cloning process

Specimens showing evidence for infection with more than one HPV-type, PCR-products were cloned in pGEMTeasy vector (©Promega, Mannheim, Germany). For each specimen, DNA extracts from 10 independent bacterial colonies were re-tested for the presence of HPV as described before.