Oral Oncology xxx (2013) xxx–xxx

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

Moritz F. Meyer [a,*,1], Christian U. Huebbers [b,1], Oliver G. Siefer [b], Julia Vent [c], Iris Engbert [d], Guy D. Eslick [e], Markus Valter [f], Jens Peter Klussmann [g], Simon F. Preuss [a]

[a] Department of Otorhinolaryngology, Head and Neck Surgery, University of Cologne, Germany
[b] Jean-Uhmacher Institute, University of Cologne, Germany
[c] Department of Otorhinolaryngology, Head and Neck Surgery, University of Mannheim, Germany
[d] Department of Obstetrics and Gynecology, Evangelisches Krankenhaus Bergisch Gladbach, Germany
[e] The Whiteley-Martin Research Centre, Discipline of Surgery, The University of Sydney, New South Wales, Australia
[f] Department of Obstetrics and Gynecology, University of Cologne, Germany
[g] Department of Otorhinolaryngology, Head and Neck Surgery, University of Giessen, Germany

ARTICLE INFO

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

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

* Corresponding author. Address: Department of Otorhinolaryngology, Head and Neck Surgery. School of Medicine, University of Cologne, Kerpener Straße 62, 50937 Cologne, Germany. Tel.: +49 221 478 4755; fax: +49 221 478 6425.
E-mail address: moritz.meyer@uk-koeln.de (M.F. Meyer).
[1] These authors contributed equally to this work.

1368-8375/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
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.

Please cite this article in press as: Meyer MF et al. Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection. Oral Oncol (2013), http://dx.doi.org/10.1016/j.oraloncology.2013.10.009

Risk-factor questionnaire

In addition, history was taken in 129 female participants by a standardized, anonymized questionnaire with focus on their habits of smoking and alcohol consumption, their sexual behaviours and practices (age at first sexual intercourse, number of sexual partners, oral-genital sexual intercourse, condom use, oral hormone therapy), medical history (including medical history of HPV related disease) and questions concerning the educational level of the participants.

Statistical analysis

All data were analyzed by software SPSS 21.0 (Statistical Package for the Social Sciences city state for descriptive statistics, students t-test, Chi-2-Test). A p-value < 0.05 was considered statistically significant.

Results

Genital HPV detection

70 Female participants (54.3%) with a mean age of 35 years were tested positive for HPV-DNA in the cervix uteri. 59 female participants (45.7%) with a mean age of 32 years showed a negative result for HPV-DNA. In 94 (72.9%) of the HPV-positive females, high-risk HPV-types could be detected. HPV16 was found in 50 of these specimens (53.5%).

Oral HPV detection

In 5.4% (n = 7) of the oral specimens, HPV-DNA was detected (Table 1). Two participants (1.6%) showed a positive result for HPV in the smear of the tonsils and 5 participants (3.9%) showed a positive result in the oral lavage. None of the participants was positive for HPV in both, the tonsil smear and the oral lavage. More low-risk HPV-types (n = 4, 57.1%) were detected than high-risk HPV-types (n = 3, 42.9%).

Oral HPV prevalence in genital HPV positive female participants vs. negative female participants

Oral HPV infection in genital HPV-positive female participants was not significantly more frequent than in genital HPV-negative participants. In 4 (5.7%) of the genital positive participants an oral HPV infection was detected. In 3 (5.1%) of the genital negative participants we found a HPV positive oral result. In one female (0.8%), we could detect the same HPV type (HPV type 54) in the cervix and the oral cavity.

Detection of HPV in 15 heterosexual couples

In addition, we analyzed 30 specimens of 15 heterosexual, monogamous couples. None of the 30 oral specimens was tested HPV positive. About 33% (n = 5) of the female participants had a positive HPV smear of the cervix (80% high grade HPV) (n = 4).

Table 1
HPV-types detected in oral samples of the study participants.

Oral HPV-positive detection 5.4% (n = 7)	Positivity in smear of the tonsils 1.6% (n = 2)	Positivity in oral lavage 3.9% (n = 5)
Low-risk (LR)-HPV 3.1% (n = 4)	HPV 54	HPV 54, 2x HPV 84
High-risk (HR)-HPV 2.3% (n = 3)	HPV 52	HPV 18, HPV 56

Analysis of the questionnaire

We analyzed potential sexual risk factors for HPV infection. The group of female participants with a positive uterine cervical HPV infection was significantly younger (32 years vs. 37) (p = 0.02) than the group of participants with a negative HPV status (Table 2). Female participants with HPV colonization in the uterine cervix had significantly more lifetime sex-partners than female participants without genital HPV detection (p = 0.001). The age of first sexual contact was a risk factor for HPV-infection (p = 0.45). Genital HPV positive participants were significantly more often smokers (p = 0.001). Between the group of genital high risk HPV infection and low risk HPV infection there were no significant differences concerning the criteria named above. Analyzing the results of the questionnaire, we could not identify any risk factors for oral HPV infection. Neither smoking habits, nor alcohol consumption, nor sexual practices (including oral sexual relation) were found to significantly influence oral HPV status (Table 3).

Discussion

Since 1983, it has been known that HPV is an important risk factor for developing carcinomas of the uterine cervix with a HPV-detection rate of almost 100% in these cases [3]. In addition, the causal role of high-risk HPV in the carcinogenesis of head and neck squamous cell carcinoma has been shown in recent studies. Especially in oropharyngeal carcinoma (OSCC), HPV was shown to be present in about 30–40% of all cases [9,14,15]. The group of patients with an HPV-associated OSCC differs from HPV-negative patients in regard to the pathogenesis, risk factors and the prognosis and thus represents a distinct tumor entity. High-risk HPV16 plays the most important role in the carcinogenesis of these cancers [10]. However, it is still unclear in which way HPV infects the oropharyngeal squamous cells. It has been discussed that particularly the tonsils are suitable for a HPV-infection in regard to the special composition of the squamous epithelium. In contrast to other epithelium in the head and neck region, the tonsils are covered with parts of single layer squamous cell epithelium (basal cell layer). Furthermore, this basal cell layer contains undifferentiated cells which might be more vulnerable to HPV [12,31].

In general, three methods are used for the detection of HPV in clinical diagnosis and biomedical research, i.e. polymerase chain reaction (PCR)-based methods, fluorescence in situ hybridization (FISH) and immunohistological staining against the surrogate marker $p16^{INK4A}$. FISH is a sensitive method, but requires predefined knowledge of the HPV-type to be detected. $P16^{INK4A}$ immunostaining has long been identified as an objective biomarker for HR-HPV-positive (pre)malignancies of the uterine cervix, allowing the unambiguous identification of truly dysplastic cells in biopsies. However, immunostaining is not suitable for non-malignant HPV-positive cells and LR-HPV induced malignancies. PCR is a highly sensitive method applicable on various types of tissue and previous studies showed that, combined with the methods of oral cell extraction (cytobrush/oral lavage), results for both methods are comparable in regard to the DNA quality and length [32–34].

The most important way of viral transmission for the genital HPV is sexual intercourse. There is good evidence that male partners play an important role in transmission of the virus in respect of their female partners [1]. In a recent study 23 male partners were tested for genital HPV and their female sexual partners were HPV-infected. In 56.5% of the couples (13/23) a concordance of at least one viral subgroup of HPV was detected. Therefore, it was

Please cite this article in press as: Meyer MF et al. Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection. Oral Oncol (2013), http://dx.doi.org/10.1016/j.oraloncology.2013.10.009

Table 2
Genital HPV-infection and potential risk factors in the study participants.

	Smear of the cervix HPV-negative ($n = 59$)	Smear of the cervix HPV-positive ($n = 70$)	p-Value
Mean age of the subjects (years)	37.5	32.6	0.03
Age at first sexual intercourse (years)	17.7	17.2	0.45
Number of lifetime sexual partners	4.55	8.71	0.001
Education duration (years)	14.83	14.69	0.87
Current smoker (%)	13.6 ($n = 8$)	47.1 ($n = 33$)	<0.001
Past smoker (%)	47.5 ($n = 28$)	75.7 ($n = 53$)	0.001
Use of alcohol, regularly (%)	30.5 ($n = 18$)	40.0 ($n = 28$)	0.20
Use of alcohol, occasionaly (%)	37.3 ($n = 22$)	47.1 ($n = 33$)	0.17
Practices oral sex (%)	62.7 ($n = 37$)	57.1 ($n = 40$)	0.31
History of warts or condylomata in general (%)	52.5 ($n = 31$)	40.0 ($n = 28$)	0.05
Skin warts (%)	3.4 ($n = 2$)	0 ($n = 0$)	0.26
Hand warts (%)	18.6 ($n = 11$)	10.0 ($n = 7$)	0.18
Foot warts (%)	33.9 ($n = 20$)	28.6 ($n = 20$)	0.34
Genital warts (%)	8.5 ($n = 5$)	4.3 ($n = 3$)	0,24
History of positive PAP-smear (%)	15.3 ($n = 9$)	47.1 ($n = 33$)	0.001
History of positive cervix dysplasia (%)	3.4 ($n = 2$)	15.7 ($n = 11$)	0.03
Use of condoms, regularly (%)	20.3 ($n = 12$)	24.3 ($n = 17$)	0.40
History of konisation (%)	8.5 ($n = 5$)	4.3 ($n = 3$)	0.16

Table 3
Oral HPV-infection and potential risk factors in the study participants.

	Oral HPV negative pat. ($n = 122$)	Oral HPV positive pat. ($n = 7$)	p-Value
Mean age of the subjects (years)	35.3	34.3	0.83
Age at first sexual intercourse (years)	17.5	16.4	0.49
Number of lifetime sexual partners	6.4	5.6	0.79
Education duration (years)	14.8	15.4	0.77
Current smoker (%)	32.0 ($n = 39$)	28.6 ($n = 2$)	0.64
Past smoker (%)	64.8 ($n = 79$)	28.6 ($n = 2$)	0.09
Use of alcohol, regularly (%)	35.2 ($n = 43$)	42.9 ($n = 3$)	0.49
Use of alcohol, occasionaly (%)	42.6 ($n = 52$)	42.9 ($n = 3$)	0.64
Practices oral sex (%)	59.0 ($n = 72$)	71.4 ($n = 5$)	0.34
History of warts or condylomata in general (%)	45.1 ($n = 55$)	57.1 ($n = 4$)	0.42
Skin warts (%)	1.6 ($n = 2$)	0 ($n = 0$)	0.92
Hand warts (%)	13.9 ($n = 17$)	14.3 ($n = 1$)	0.55
Foot warts (%)	32.0 ($n = 39$)	14.3 ($n = 1$)	0.50
Genital warts (%)	4.1 ($n = 5$)	42.9 ($n = 3$)	0.03
History of positive PAP-smear (%)	32.0 ($n = 39$)	42.9 ($n = 3$)	0.56
History of positive cervix dysplasia (%)	9.0 ($n = 11$)	28.6 ($n = 2$)	0.15
Use of condoms, regularly (%)	23.0 ($n = 28$)	14.3 ($n = 1$)	0.62
History of konisation (%)	5.7 ($n = 7$)	14.3 ($n = 1$)	0.55

hypothesized that oral HPV-infection had to have the same mechanisms of viral transmission. A possible way could be the transmission of the virus by oral sexual contact or a self inoculation [10,35].

We detected oral HPV-infection in 5.4% of all female participants. These results are in line with a study of Summersgill et al. [36], who reported a HPV infection rate of 5.2% in female and male adolescents [36]. Nevertheless, the group of participants with positive cervical HPV smear was not more often infected by HPV in the oral cavity/tonsil area ($n = 4$, 5.7%) than the genital HPV-negative group ($n = 3$, 5.1%). Only in one case (0.8%) we could detect the same HPV low risk subgroup in specimens of the cervix uteri and in specimens of the oral cavity (HPV 54). In accordance to these results, several authors also could show a concordance between HPV-infection of the cervix and the oral cavity [35,37]. Similar to our study, Smith et al. [10] showed in only 1% of all female participants an HPV-detection in the oral cavity and the cervix at the same time. In that study, none of the simultaneously HPV-infected participants showed the same subtype of HPV in the cervical and oral specimens. Even in present studies of high risk patients (HIV-positive) a concomitant infection at the two sites (oral cavity and cervix uteri) could not be identified [38].

The HPV-genotype spectrum in the oral mucosa was similar to that encountered in the cervix uteri specimens. The detection of oral low grade HPV was possible in 4 of all HPV-positive participants (57.1%) and in 3 of all HPV-positive cases (42.9%), high-risk HPV was detected. In other studies it was reported that HPV16 was the single most frequent genotype in oral mucosa, followed by multiple type infections [28,39]. The finding that low-risk HPV types are more frequent in oral samples than in cervical samples is in agreement with previous reports [39].

In our small study sample, there is a direct correlation of the number of sexual lifetime partners and genital HPV-infection (HR- and LR-HPV, $p = 0.001$). However, no correlation could be shown between sexual lifetime partners and the infection rate of the oral cavity by HPV ($p = 0.79$) (Table 3).

Oral sexual transmission of HPV from man to woman and woman to man has often been discussed among the risk factors of oral HPV-infection and is still under debate [10,29]. This could not be confirmed in the present study but with limited data due to a small sample size. The oral positive HPV group stated to have more oral sexual relation in the questionnaire but a statistical significance could not be shown ($p = 0.34$) (Table 3). We could not detect a correlation between the oral HPV infection rate and smoking habits. About 30% ($n = 41$) of all HPV-positive and negative participants were frequent smokers at time of the study ($p = 0.64$) (Table 3).

Furthermore, we could not find a relation of oral and genital HPV-infection analyzing 15 heterosexual couples. None of the male and the female participants showed a positive oral result for HPV. In the study of Smith et al. 69 male participants had an incidence

Please cite this article in press as: Meyer MF et al. Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection. Oral Oncol (2013), http://dx.doi.org/10.1016/j.oraloncology.2013.10.009

for oral HPV-detection of 5.9% [37]. But in this study, the female partners had no genital HPV infection.

A possible explanation for the missing correlation between genital and oral HPV-infection could be the different types of virus clearing-possibilities depending on the squamous cell area [9,35]. Similar to the cervix uteri, the oral cavity lining consists of mucosal epithelium. It was hypothesized that antimicrobial components of the saliva, e.g. lysozyme, lactoferrine, immuneglobuline A und cytokines have a protective impact amongst others against viral agents. Therefore the contact time between the virus and the oral mucosa might be reduced [35]. But if these components prevent a persisting HPV infection is still unclear.

A vaccination against HPV has been proposed not only for women but lately also for men [1]. If the prophylactic HPV-vaccination reduces the burden of HPV-related diseases in both cervix and oropharynx will be seen in the future.

In conclusion, the results in this study add to the hypothesis that there is less concordance between the HPV infection in the cervix and the oral cavity/tonsils than expected. In contrast to vaginal sexual intercourse as a main risk factor for carcinoma of the uterine cervix, oral sexual practice seems to have less influence to the transmission of HPV to the oral cavity. The results of our study must however be interpreted carefully given the small sample size of our study cohort. Larger cohorts should be tested to confirm these preliminary data.

Conflict of interest statement

Any financial and personal relationships with other people or organisations that could inappropriately influence (bias) the work are disclosed by all authors. The authors declare that they have no conflict of interest.

References

[1] De Lima Rocha MG, Faria FL, Gonçalves L, Souza Mdo C, Fernandes PÁ, Fernandes AP. Prevalence of DNA-HPV in male sexual partners of HPV-infected women and concordance of viral types in infected couples. PLoS ONE 2012;7(7):e40988. http://dx.doi.org/10.1371/journal.pone.004098. Epub 2012 Jul 17.

[2] Hernandez BY, Wilkens LR, Zhu X, Thompson P, McDuffie K, Shvetsov YB, et al. Transmission of human papillomavirus in heterosexual couples. Emerg Infect Dis 2008;14(6):888–94.

[3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189(1):12–9.

[4] Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002;55(4):244–65.

[5] Wittekindt C, Wagner S, Mayer CS, Klussmann JP. Basics of tumor development and importance of human papilloma virus (HPV) for head and neck cancer. GMS Curr Top Otorhinolaryngol Head Neck Surg 2012;11:Doc09. http://dx.doi.org/10.3205/cto00009. URN: urn:nbn:de:0183-cto0000912.

[6] Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. Cancer 1997;79(3):595–604.

[7] Pfister H. The role of human papillomavirus in anogenital cancer. Obstet Gynecol Clin North Am 1996;23(3):579–95.

[8] Snijders PJ, Cromme FV, van den Brule AJ, Schrijnemakers HF, Snow GB, Meijer CJ, et al. Prevalence and expression of human papillomavirus in tonsillar carcinomas, indicating a possible viral etiology. Int J Cancer 1992;51(6):845–50.

[9] Preuss SF, Klussmann J-P, Semrau R, Huebbers C. Update of HPV-induced oropharyngeal cancer. HNO 2011;59(10):1031–7.

[10] Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, et al. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer 2004;108(5):766–72.

[11] D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356(19):1944–56.

[12] Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Eckel HE, Pfister HJ, et al. Human papillomavirus-positive tonsillar carcinomas: a different tumor entity? Med Microbiol Immunol 2003;192(3):129–32.

[13] Lindel K, Beer KT, Laissue J, Greiner RH, Aebersold DM. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. Cancer 2001;92(4):805–13.

[14] Klussmann JP, Weissenborn SJ, Wieland U, Dries V, Kolligs J, Jungehuelsing M, et al. Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. Cancer 2001;92(11):2875–84.

[15] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14(2):467–75.

[16] Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol 1998;153(6):1741–8.

[17] Herrero R, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaram P, et al. Human papillomavirus and oral cancer: the international agency for research on cancer multicenter study. J National Cancer Inst 2003;95(23).

[18] Steinberg BM, DiLorenzo TP. A possible role for human papillomaviruses in head and neck cancer. Cancer Metastasis Rev 1996;15:91–112.

[19] Olthof NC, Straetmans JM, Snoeck R, Ramaekers FC, Kremer B, Speel EJ. Next-generation treatment strategies for human papillomavirus-related head and neck squamous cell carcinoma: where do we go? Rev Med Virol 2012;22:88–105.

[20] van Houten VM, Snijders PJ, van den Brekel MW, Kummer JA, Meijer CJ, van Leeuwen B, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. Int J Cancer 2001;15(93):232–5.

[21] Wiest T, Schwarz E, Enders C, Flechtenmacher C, Bosch FX. Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. Oncogene 2002;28(21):1510–7.

[22] Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 1989;115(5):621–5.

[23] Tsuchiya H, Tomita Y, Shirasawa H, Tanzawa H, Sato K, Simizu B. Detection of human papillomavirus in head and neck tumors with DNA hybridization and immunohistochemical analysis. Oral Surg Oral Med Oral Pathol 1991;71(6):721–5.

[24] Franceschi S, Munoz N, Bosch XF, Snijders PJ, Walboomers JM. Human papillomavirus and cancers of the upper aerodigestive tract: a review of epidemiological and experimental evidence. Cancer Epidemiol Biomarkers Prev 1996;5(7):567–75.

[25] Klussmann JP, Gultekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 2003;162(3):747–53.

[26] Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709–20.

[27] Pintos J, Black MJ, Sadeghi N, Ghadirian P, Zeitouni AG, Viscidi RP, et al. Human papillomavirus infection and oral cancer: a case-control study in Montreal. Canada Oral Oncol 2008 Mar;44(3):242–50.

[28] Kreimer A, Bhatia RK, Messeguer AL, Gonzalez P, Herrero R. Oral human papillomavirus in healthy individuals: a systematic review of the literature. Sex Transm Dis 2010;37:386–91.

[29] D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009;199:1263–9.

[30] Giovannelli L, Lama A, Capra G, Giordano V, Arico P, Ammatuna P. Detection of human papillomavirus DNA in cervical samples: analysis of the new PGMY-PCR compared to the hybrid capture II and MY-PCR assays and a two-step nested PCR assay. J Clin Microbiol 2004;42:3861–4.

[31] Mischke D, Genka T, Wille G, Lobeck H, Wild AG. Keratins as molecular markers of epithelial differentiation: differential expression in crypt epithelium of human palatine tonsils. Ann Otol Rhinol Laryngol 1991;100(5 Pt 1):372–7.

[32] Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 2001;10(6):687–96.

[33] King IB, Satia-Abouta J, Thornquist MD, Bigler J, Patterson RE, Kristal AR, et al. Buccal cell DNA yield, quality, and collection costs: comparison of methods for large-scale studies. Cancer Epidemiol Biomarkers Prev 2002;11(10 Pt 1):1130–3.

[34] Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I. Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection. J Oral Pathol Med 1992;21(6):265–9.

[35] Termine N, Giovannelli L, Matranga D, Perino A, Panzarella V, Ammatuna P, et al. Low rate of oral human papillomavirus (HPV) infection in women screened for cervical HPV infection in Southern Italy: a cross-sectional study of 140 immunocompetent subjects. J Med Virol 2009;81:1438–43.

[36] Summersgill KF, Smith EM, Levy BT, Allen JM, Haugen TH, Turek LP. Human papillomavirus in the oral cavities of children and adolescents. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91(1):62–9.

[37] Smith EM, Ritchie JM, Yankowitz J, Wang D, Turek LP, Haugen TH. HPV prevalence and concordance in the cervix and oral cavity of pregnant women. Infect Dis Obstet Gynecol 2004;12(2):45–56.

[38] Gillison M. Relationship between prevalent oral and cervical human papillomavirus infections in human immunodeficiency virus–virus-positive and -negative women. J Clin Microbiol 2006;44:4479–85.

[39] Rautava J, Willberg J, Louvanto K, Wideman L, Syrjanen K, Grenman S, et al. Prevalence, genotype distribution and persistence of human papillomavirus in oral mucosa of women: a six year follow-up study. PLoS ONE 2012;7(8):e42171.

Please cite this article in press as: Meyer MF et al. Prevalence and risk factors for oral human papillomavirus infection in 129 women screened for cervical HPV infection. Oral Oncol (2013), http://dx.doi.org/10.1016/j.oraloncology.2013.10.009