PCR Technique Assisting the Early Diagnosis of Human Papillomavirus

A retrospective clinical study

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The number of oral cancers associated with Human Papillomavirus (HPV) infection is increasing worldwide. The purpose of this retrospective clinical trial is to investigate the relationship between genital and oral HPV infection and to observe the possibilities of polymerase chain reaction (PCR) technique and the limitations for oral HPV infection. The results of genital and oral HPV screening of 34 female patients and their partner, and 14 single female patients were processed in a private practice in Budapest between 2012 and 2015. After brush-biopsy sampling, HPV DNA identification and typing were performed using PCR technique. All in all 164 samples were typed. HPV DNA was detected in 76 cases (48.20%). HPV was detected in 55 cases from the genital samples (67%), and in 21 cases from the oral samples (25.6%). Genital HPV infection was statistically significantly higher in women (79.2% vs 22,9%, $p<0.001$). Gender comparison of genital HPV infection showed a statistically significant difference (79.2% for women, 50% for men, $p = 0.006$). In case of genital HPV infection, the oral HPV infection of the same person is more frequent. In this study, the above mentioned difference is significant for women and not significant for men. HPV16 is the HPV genotype which was identified in the highest ratio(47.2%). PCR technique is capable of the detection of oral HPV infection, but follow-up studies with higher case number , and questionnaire studies are needed to understand HPV transmission more accurately.

Keywords: polymerase chain reaction, oral cancer, human papillomavirus

Human papillomavirus (HPV) infection is the most common sexually transmitted disease in the world [1]. HPV infection is diagnosed in approximately 6 million cases annually and 9.0-13.0 per cent of the world population are infected. More than 100 genotypes of HPV are known. There are low-risk groups (Low-Risk HPV, LR HPV), such as HPV 6,11, which play a role in the aetiology of benign lesions such as papilloma or condyloma acuminatum. There are high-risk, oncogenic genotypes (High-Risk HPV, HR HPV), such as HPV16,18 and 31, which are responsible for the development of malignant lesions (e.g., cervix carcinoma, oropharyngeal tumours). Worldwide 6.7 per cent of all malignant tumours is due to oncogenic HPV infection [2]. Malignant tumours associated with HPV have a highest incidence on the cervix, there 99.7 per cent of malignant lesions have oncogenic HPV infection in the background [3]. Oncogenic genotypes have been isolated from cancers of the vulva, vagina, penis, anus and oropharyngeal and oesophageal cancers. Low-risk genotypes HPV6 and HPV11 are most often present in the mucus membranes causing papilloma and condyloma acuminatum [4].

In Hungary according to the data of the Hungarian Central Statistical Office the incidence of cervix carcinoma is around 1500 cases per year and around 500 patients die annually [3]. Hungary is the leader in incidence and mortality in oropharyngeal cancer in Europe and is second in the world in terms of men [5] . In terms of the numbers in 2014 according to the National Cancer Registry 3765 new cases of head and neck cancer have been registered, 2688 men and 1077 women. According to data from the Hungarian National Statistical Office in 2015 1157 male and 303 female patients died of head and neck cancer.

The aetiology of oral cancer is multicausal. A distinction between HPV positive and HPV negative tumours is made in the literature. Classic etiologic factors include smoking, excessive alcohol consumption, male gender, old age, poor oral hygiene and mechanical irritation [6]. Due to current sexual behaviour oral HPV infection is becoming more and more common [7]. In the past 30 years the number of HPV positive oral cancers has been on the rise [8, 9], especially in the case of tonsil tumours, in 70 per cent of which oncogenic HPV can be detected [10, 11]. The prevalence of smoking has a decreasing tendency worldwide as well as in Hungary, but the incidence of oral cancer is stagnating. Oncogenic HPV-positive tumours are probably the explanation for this discrepancy [8, 12]. Characteristics of HPV related tumours include: location in the posterior segment of the mouth (posterior tongue, soft palate, tonsils, mesopharynx), the male:female ratio is typically 1:1, patients are younger, presence of precancerous lesion is rare. The prognosis of HPV-positive cancers is better, recurrence is less frequent and they respond better to radiotherapy [13]. The etiologic role of the oncogenic HPV 16 genotype is proven in oral cancer. HPV 16 isolated from the back of the mouth can be considered a 13 fold risk factor [14].

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HPV screening plays a very important role in the prevention of cervix carcinoma as it successfully reduces the incidence as well as the mortality of the disease [2]. Screening for oral cancer involves an annual dental checkup visit where a thorough extraoral and intraoral examination could identify suspect lesions. These are then biopsied in oral surgery departments to provide a final diagnosis. There are tools to aid the diagnosis of lesions invisible to the naked eye [15], such as the VELscope based on the principles of autofluorescence that can help differentiating between healthy mucosa, hyperkeratotic mucosa and dysplastic mucosa. These tools are not very widespread in dental practice yet, but it has to be stressed that almost 90 per cent or oral malignancies can be identified through inspection [16]. The increasing proportion of HPV-positive oral tumours requires HPV screening similar to gynaecology. Based on the literature, similarly to genital samples, a simple, non-invasive brush biopsy and subsequent DNA Polymerase Chain Reaction (PCR) are accepted as a means to detect and determine the type of oral HPV infection [7, 17, 18].

The goal of the current paper is the retrospective study of the genital and oral HPV screening of couples and female patients presenting to a private practice in Budapest offering dental and gynaecological care between 2012 and 2015. Correlation between genital and oral HPV will be studied.

Experimental part

Materials and methods

The data presented in this study are from a selection of patients who presented to a private practice in Budapest between 2012 and 2015. In patients presenting for routine gynaecological screening clinical and cytological examinations were conducted as well as HPV typing. Following this the patients requested screening for oral cancer and HPV and, if they had a current partner then their genital (glans penis, corona of glans penis, external urethral meatus) and oral screening for cancer and HPV. Female patients who had any pathology on colposcopy or cytology, who had a history of chemotherapy or radiotherapy, who were immunosuppressed or received aspecific immunostimulant treatment (Inosine Pranobex) or who had been vaccinated for HPV were excluded from the study. Similarly, male patients with any genital abnormality, a history of chemo- or radiotherapy, immunosuppression, aspecific immunostimulant treatment (Inosine Pranobex) or HPV vaccination were not included in the study. A total of 34 couples and 14 female patients not living in a relationship were enrolled in the study. In the case of couples the females' mean age was 30.3 years (19-60 years), that of males was 35.7 years (21-66 years), the mean age of ladies not living in a relationship was 28.9 years (22-40 years). Patients were informed of the possibility of oro-genital transmission of HPV and the details of the examinations and the study and informed consent was obtained. One and the same practitioner performed the genital examination in all cases and the second practitioner performed all the oral examinations and HPV sample collection. Oral examination involved clinical screening for any mucosal lesion then a brush biopsy was performed. The exfoliated cells collected were placed into a transport medium of 1 mL PBS (phosphate buffered saline) and were stored at -20°C until further examination. Collection of genital and oral samples were done at the same time for partners or within a week.

Sequences specific to HPV in clinical samples were identified in the Department of Medical Microbiology of the University of Debrecen. The exfoliated cells were prepared for nucleic acid isolation with the following method: 1 mL cell suspension was centrifuged at room temperature for 5 min at 500 g and the sediment was suspended in 200 μL PBS solution. DNA isolation was don with the help of the innuPrep Viral RNA/DNA kit (Analytik Jena, Jena, Germany) following the manufacturer1s protocol. Briefly, 200μL Carrier mix-containing CBV lysis buffer and 20 μL proteinase K were added to 200 μL of cell suspension, and it was incubated at 70°C for 10 min. Following incubation 400 μL SBS binding buffer is added to the samples and vortexed and then was added to the Spin Filter column and centrifuged for 1 min at 10000 g. Following this the columns were washed with once 500 μL HS, and twice with 650-650 μL LS buffer and between each step the samples were centrifuged for 1 min at 10000 g. After this the columns were further centrifuged for 5 minutes at 10000 g to remove the buffer. Nucleic acid was eluted from the columns with 60 μL of RNAse-free water preheated to 70°C; the columns were incubated at room temperature for 2 min and then centrifuged at 8000 g for one min. DNA was stored at -20°C until further processing.

The quality of the DNA was checked using a polymerase chain reaction (PCR) specific to the human β-globin gene. HPV-specific sequences were detected using MY/GP consensus nested PCR that is specific to the conservative L1 gene of mucosal HPV that is usable to detect these HPV genotypes [19]. HPV genotype detection was done either with restriction fragment length polymorphism (RFLP) of MY PCR products [20] or with the sequencing of GP amplimers (Macrogen, Amsterdam, The Netherlands). In cases when a mixed infection was suspected and the MY PCR yielded a positive result, genotypes were identified with the help of a GenoFlow HPV array test kit (DiagCor, Kowloon Bay, Hong Kong) following the manufacturer's protocol. In the case of HPV6, HPV11, HPV16, HPV18, HPV31 and HPV33 genotypes the results of genotyping were confirmed with type-specific PCR as well[21]. In a few cases HPV genotyping was unsuccessful due to the low number of copies. These cases were classified as slightly positive, not identifiable (NI).

In statistical calculations frequency tables were used to demonstrate genital and oral HPV prevalence in women and men and the prevalence of HPV infection in partners was also analysed in couples. The various groups were analysed using the Chi square and Fisher's exact tests. For the statistical analysis we used IBM SPSS statistics 23 software (IBM Corporation).

Results and discussions

The stomatooncological screening of 82 patients did not reveal any precancerous or malignant lesion.

A total of 34 couples had genital and oral HPV screening resulting in 136 samples. An additional 14 female patients had genital and oral HPV screening who did not live in a relationship. This yielded another 28 samples. Thus a total of 164 samples were studied. HPV DNA was demonstrated in 76 cases (46.30%). In females presenting with their partners HPV DNA was detected in 28 genital samples (82.40%), in females without a partner 10 cases were positive for HPV DNA from genital samples (71.40%), thus HPV DNA was detectable in a total of 34 out of 48 female genital samples (79.20%). Out of the 34 male patients HPV DNA was detectable in 17 genital samples(50%). *Genital HPV infection showed a statistically significant difference ($p<0.01$) between the two sexes* (table 1).

In 21 of the oral samples HPV DNA was detected (25.60%), in 9 of the ladies with partners (26.50%), and in 2 of the ladies not in a relationship (14.30%), thus 11 females

	MALE (N=34)		FEMALE (N=48)		P VALUE
MEAN AGE	35.74		29.9		
HPV GEN +	17	50%	38	79.20%	0.006 *
HPV ORAL +	10	29.40%	11	22.90%	0.507

Table 1
GENITAL AND ORAL HPV INFECTION RATE BASED BY SEX. Significant differences are marked with *

	HPV GENITAL POSITIVE		HPV ORAL POSITIVE		P VALUE
FEMALE	38	79.2%	11	22.9%	<0.001 *
MALE	17	50%	10	29.40%	0.08

Table 2
HPV INFECTION ACCORDING TO LOCALISATION IN FEMALES AND MALES. Significant differences are marked with *

	MALE HPV GEN- (N=17)		MALE HPV GEN+ (N=17)		P VALUE
HPV ORAL +	4	23.50%	6	35.30%	0.567
PARTNER HPV ORAL+	3	17.60%	6	35.30%	0.438
PARTNER HPV GENITAL +	14	82.40%	14	82.40%	1
	FEMALE HPV GENITAL- (n=10, couple n=6)		**FEMALE HPV GEN+ (n=38), (*Female HPV genital in couple n=28)***		**P VALUE**
HPV ORAL +	0	0%	11 *(9)*	28.90% *(32.14%)*	0.089 *(0.162)*
PARTNER HPV ORAL+	3	50%	7	25%	0.328
PARTNER HPV GENITAL +	3	50%	14	50%	1

Table 3
HPV TRANSMISSION STUDIES BETWEEN MALES AND FEMALES. Significant differences are marked with *

(22.90%), and 10 males (29.40%) were infected. It was found that genital HPV infection was present in a higher percentage both in women and in men than oral infection (table 2), which difference was significant in the case of women and not significant in the case of men (79.2% vs 22.9%, $p<0.001$).

It could be seen that in the case of patients with genital HPV infection oral HPV infection is more common than in the case of no genital HPV infection. This difference is 32.14% *vs.* 0% in females and 35.50% *vs.* 23.50% in males (table 3.), which is not significant statistically.

In terms of oro-genital transmission it could be seen (table 3) that the partners of males with a genital HPV infection had a higher percentage of oral HPV infection than those of genitally HPV negative males (17.60% *vs.* 35.30%, $p=0.438$). In the case of women, however, surprisingly, the partners of genitally HPV negative women had a higher rate of oral HPV infection than partners of HPV positive women (50% *vs.* 25%, $p= 0.328$), although the difference was not significant. In terms of genital transmission there was no difference between the partners of HPV positive vs. negative women (50% *vs.* 50%) or HPV positive vs. negative men (82.40% *vs.* 82.40%) either as far as genital HPV infection is concerned.

There is statistically significant difference ($p=0.023$) between men and women, however, in terms of the HPV infection of the partner of genitally HPV positive patients (table 4.): the genital infection rate of the partners of males was 82.4% (i.e. the genital HPV infection of female partners), for women it was 50% (genital HPV infection of male partners). This was similar in oral samples as well, but the difference was not significant (35.30% vs 25%, $p=0.461$).

The 76 samples positive to HPV DNA show the following genotype distribution: in the case of 5 samples LR HPV has been identified (genotypes 11, 53, 57, 61, 81), in a total of 27 cases HR HPV monoinfection has been detected, out of which 15 were the genotype HPV16, 3 were HPV 56 and another 3 were HPV 66, the other 6 HRs were 18, 31, 33, 45, 51, 58. A mixed infection was seen in four cases: HPV 16/6, 45/68, 16/51, 31/39/45, all of which contain an HR genotype. HPV was not identifiable from 40 samples (HPV NI).

	HPV GENITAL POSITIVE PATIENTS				
	male (n=17)		female (n=28)		p value
HPV GEN + PARTNER	14	82.4%	14	50%	0.023 *
HPV ORAL + PARTNER	6	35.30%	7	25%	0.461

Table 4
COMPARISON OF THE HPV INFECTION OF PARTNERS OF GENITALLY HPV POSITIVE MEN AND WOMEN. Significant differences are marked with *

The distribution of HPV LR, HPV HR, HPV 16 and HPV NI genotypes according to infection site and gender is shown in table 5.

Examining the genotypes it was found that out of the 38 genital HPV infections in women 23 had HR HPV (60.50%), out of which HPV 16 DNA (44%) was identified in 10 cases, which is the most common of the identified genotypes. In males with genital HPV DNA positivity HR HPV-DNA was identified in 4 cases (23%), out of which 3 were HPV 16 (75%). Out of the 21 HPV positive oral samples 4 (5.25%) were found to be HR HPV, all of which was HPV 16 (100%). Two of these patients were male and two female. LR HPV DNA was only found in genital samples, in a total of 5 cases, 4 in women (10.5%) one in a man (5.9%). Out of the 76 HPV DNA positive samples the genotype could not be identified (HPV NI) in 40 cases (52%), the lowest ratio (29%) was amongst female genital samples (n=11), the highest ratio (81.8%) was amongst female oral samples (n=9).

PCR Technique is an important element in assisting the early diagnosis of human papillomavirus and also in the diagnosis and the treatment of periodontal disease. [32] The causative aetiological role of HPV in cervical cancer has been known since 1983 and HPV can be detected in almost 100% of the malignancies in the region [2]. High risk HPV DNA can integrate into mucosal cells, thus viral genes E1 and E2 are damaged. These two genes are responsible for the regulation of the expression of viral oncogenes E6 and E7 which play a role in the inhibition of cellular tumour suppressor genes (p53, pRB). The outcome is the over expression of the oncoproteins E6 and E7 can leads to the malignant transformation of the infected cell [22] . Due to changed sexual habits HPV appears in the

Table 5
HPV INFECTION ACCORDING TO SEX AND LOCALISATION. In the HR HPV group HPV 16 is shown in parenthesis

	LR HPV		HR HPV (HPV16)		HPV NI		Significant difference between the groups
	Count	N %	Count	N %	Count	N %	
Female-couple-Genit, (n=28)	3	10.7%	17 (6)	60.7% (21.4%)	8	28.6%	a
Female-couple-Oral, (n=9)	0[1]	0.0%	1 (1)	11.1% (11.1%)	8	88.9%	b
Female-alone-Genit, (n=10)	1	10.0%	6 (4)	60.0% (40.0%)	3	30.0%	-
Female-alone-Oral,(n=2)	0[1]	0.0%	1 (1)	50.0% (50.0%)	1	50.0%	-
Female-total-Genit (n=38)	4	10.5%	23 (10)	60.5% (26.3%)	11	28.9%	a, b
Female-total-Oral (n=11)	0[1]	0.0%	2 (2)	18.2% (18.2%)	9	81.8%	b
Male - Genit, (n=17)	1_a	5.9%	4 (3)	23.5% (17.6%)	12	70.6%	b, c
Male - Oral, (n=10)	0[1]	0.0%	2 (2)	20.0% (20.0%)	8	80.0%	b

Comparison of genotype distributions within each group using the Bonferroni correction.
a means significant difference between LR HPV and HR HPV groups.
b means significant difference between HR HPV and NI HPV groups.
c means significant difference between LR HPV and NI HPV groups
1.This category is not used in comparisons because its column proportion is equal to zero or one.

oral cavity via oro-genital transmission, where it persists or can cause the development of benign of malignant lesions. The lesions with HPV as an aetiological factor in the oral cavity can be benign, such as papilloma, condyloma acuminatum, precancerous lesions, such as leukoplakia, or malignant, squamous cell carcinoma. HPV 16 is thought to be responsible for the development of HPV related malignancies. Kreimer et al. (2010) concluded in their review that HPV DNA could be identified in 35.6% of oropharyngeal tumours, out of which 87% could be identified as HPV 16 [23]. The presence of the HPV 16 genotype in the oral cavity can be considered a risk factor of oral malignancy, similar to smoking that can increase the risk of oral cancer by 13 times [14]. In the current study, HPV 16 was the most often identified out of the high risk HPV group (HR- HPV), that could be isolated from almost half of the female genital samples (10 cases, which result is comparable to those obtained by Tatar et al [24]. Out of HR HPV genotypes HPV 16 was identified in three-fourths of the male genital samples (3 cases), and in each of the oral samples (4 cases, 2 male, 2 female).

Currently three methods are used in clinical diagnostics to identify HPV and Ebola virus desease [33]. These are methods based on polymerase chain reaction (PCR), fluorescence in situ hybridisation (FISH), and immunohistochemical staining to identify the $p16^{INK4A}$ marker [25]. FISH is a method with a sufficient sensitivity, but its disadvantage is that a DNA fragment of the HPV genotype to be identified needs to be known. The $p16^{INK4A}$ immunohistochemical stain is suitable to identify HR-HPV positive malignant lesions by demonstrating actually dysplastic cells. Its disadvantage is that it cannot identify non-malignant cells infected with HPV. It cannot be used to identify LR-HPV infection. PCR is a high sensitivity technique that can be used to study samples from a variety of tissues, which combined with oral cell extraction (cf. cytobrush) is an accepted method of oral HPV screening[26, 27]. The high sensitivity of PCRmakes it possible to identify HPV DNA present in a low copy number. Szarka et al. (2009) studied the HPV DNA copy numbers in samples from malignant oral squamous cell tumours, oral precancerous lesions and from exfoliated cells of clinically healthy oral mucosa from the same patients as well as from healthy individuals and found that the copy number of the virus had a correlation with the severity of the lesions [19]. Besides they also studied the number of copies of HPV DNA in genital and oral samples and found that in women suffering from HSIL the copy of the virus DNA was significantly higher in the genital tract than in their partners' genital samples, but the viral load of the oral mucosa did not show a significant difference according to sex[24]. It is important to determine what viral load has a clinical relevance and a prognostic value in predicting the development and prognosis of oral lesions, which would necessitate further long term follow-up studies in large populations.

In the present study the correlation between genital and oral HPV infection was studied in the case of 34 couples and 14 single women. Out of the 48 female participants HPV DNA was identified in the genital sample in 38 cases (79.20%) and a total of 11 cases (22.90%) of oral HPV positivity were identified, therefore genital HPV infection in women is significantly more common than oral HPV infection This is in line with the data published by Meyer et al.[25], who compared the genital and oral HPV status of 129 women and found that 54.3% had genital HPV DNA positivity 5.4% had oral HPV infection. Almost a third of HPV positive oral samples originated from women with genital HPV infection and no HPV DNA was found in the oral samples of women who had no genital HPV infection, which correlates with a number of studies[24, 25, 28, 29]. Meyer et al. (2014) found HPV DNA in the oral samples of 5.7% of women with genital HPV infection and in 5.1% of genitally HPV negative women. Giraldo et al. [29] compared the oral HPV status of 70 genitally HPV positive women with that of 70 genitally HPV negative women. Their results showed that oral HPV prevalence was significantly higher in genitally HPV DNA positive patients (37.1%), than in genitally HPV DNA negative patients (4.3%). Furthermore 89.7% of orally HPV infected patients had genital HPV infection, too, which was 100% in the present study. In the present study genital HPV infection was also more common in men than oral HPV infection (50% *vs.* 29.4%), though the difference is not statistically significant. In the women studied the prevalence of genital infection was significantly higher than in men. The prevalence of oral HPV infection was higher in men than women, but this difference was not statistically significant. The person with genital HPV infection had a higher chance of oral HPV infection in both genders (table 3.). This must no be forgotten in interdisciplinary communications as women participate in genital HPV screening more often than oral screening if HPV positive patients were informed of the necessity of oral screening (both for the patient and her partner) then an important risk group could undergo stomatooncological screening. Upon studying cross infection it can be stated that genitally HPV infected men's partners have a higher risk of oral infection than partners of genitally HPV negative men (35.3% *vs.* 17.6%). Furthermore partners of genitally HPV infected men had a higher ratio of genital and oral HPV infection than partners of genitally HPV infected women (table 4.). The incidence of genital lesions with an HPV related aetiology is low, just as genital HPV screening, therefore persistent cases of HPV are difficult to identify and continue to threaten the genital and oral health of their partners. Based on these considerations the routine genital screening of males for HPV and the adequate management of these cases seem to gain importance. This could theoretically reduce the number of HPV related oral malignancies. Carrying this thought further the extension of HPV vaccination to boys might have a preventative value [30].

Tatar et al. [24] found a lower percentage of genital HPV infection in males and genital infection was more common than oral infection both in male and females. The observation of the present study are in agreement with this. The lower ration of oral HPV prevalence could be due to the presence of antimicrobial components in the oral cavity eg. cytokines, lactoferrin, lysozyme, immuno-globulin A, therefore the contact time between the virus and the oral mucosa is reduced. The actual effectivity of these protected mechanisms is not yet clear [25]. The difference in genital HPV infection between sexes could be explained by the lack of the transformation zone (preferable to HPV) in men. No such zone is found in the oral cavity either. This is a zone in the cervix where the squamous epithelium of the orifice and the columnar epithelium of the cervical meet. The transformation zone (or junctional zone) develops at the movement of the meeting zone between squamous cells and columnar cells. Its extent and location are dependent on age. Physiologically this region is in constant movement, regeneration can be observed. HPV infection always happens through the epithelial wounds of this region or at the squamo-columnar junction [31]. A further explanation could be that in the male genital tract and in the oral cavity

the dynamics of HPV elimination is not entirely clear and data are scarce. In order to determine this further studies are necessary with large study populations and long follow-up times.

Meyer et al. [25] performed genital and oral HPV screening of 129 female patients, HPV DNA was found in oral samples in 7 cases (5.4%). LR-HPV had a higher ratio (n=4, 51.7%), than HR-HPV (n=3, 42.9%). In the present study oral HPV DNA was detected in a higher percentage, in 21 cases (25.60%) and 4 out of these(19%) were HR HPV (HPV 16) and 17 were HPV NI (81%). No low-risk HPV (LR-HPV) group was detected in oral samples. In most of the 76 HPV DNA positive samples (40 cases - 52%) the genotype could not be identified (HPV NI). Out of the 55 genital HPV positive samples 23 cases were HPV NI (42%), and out of the 21 oral samples 17 cases were HPV NI (81%), lowest ratio of HPV NI was found amongst female genital samples (29%). This ratio is much higher in male genital samples (71%) and even higher in oral samples where it was 80% for males and 82% for females. This result corresponds to those reported by Tatár et al.[24], which state that the average number of copies from cervical samples is at least a magnitude higher than in any other localisation that plays a basic role in genotype identification due to the sensitivity of the typing methodology. According to another explanation is mixed infection that can also limit genotype identification. As HPV NI results can potentially contain HR HPVs, clinically these should be considered high risk and should be managed accordingly (observation, recall, treatment).

In terms of couples there was a single case where the same genotype could be identified from both the genital and oral samples and it was a HPV 16 volt (3%). Meyer et al. identified the same HPV genotype from female genital and oral samples and it was a LR HPV 54 (0.8%). Apart from the single case discussed above there were two cases in the current study where HPV DNA could be identified both from female and male genital and oral samples but in both cases a genotype could only be determined from the female genital sample (HR HPV 58, and HR HPV 16), the genotype could not be identified in any of the other samples. Thus, out of the 34 couples a total of 3 cases (8.8%) were found where HPV DNA could be identified in all 4 samples. In the case of couples the differences in HPV genotype in the genital and oral samples are important in communications. It has to be stressed that differences in genotypes or in positivity do not suggest infidelity as the susceptibility to infection and the dynamics of elimination differ from person to person.

Conclusions

Genital HPV infection suggests a potentially higher risk for oral HPV infection in both sexes than in HPV negative patients.

HPV positive males have a higher risk of genital and oral infection than partners of HPV positive females. This has a preventative importance in interdisciplinary communication due to persisting infection, common in men.

Oral HPV screening can be done at the same time as stomatooncological screening and is non-invasive.

PCR routinely used in laboratory diagnostics is useful in oral HPV screening because of the lower number of HPV DNA copies than in the genital region.

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