Prevalence, Genotype Distribution and Persistence of Human Papillomavirus in Oral Mucosa of Women: A Six-Year Follow-Up Study

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Abstract

Background: Human papillomavirus (HPV) infections have been linked to a subset of oral and oropharyngeal cancers. However, little is known on the natural history of oral HPV infections. We designed the prospective Finnish HPV Family Study to assess the dynamics of HPV infections in parents and their infants. This study reports HPV genotype distribution and virus persistence in oral mucosa of the mothers.

Materials and Methods: Totally, 324 pregnant women were enrolled at the 3rd trimester of pregnancy and followed-up for 6 years. Oral scrapings taken with a brush were collected and HPV-genotyping was performed with nested PCR and Multimetrix® test (Progen, Heidelberg, Germany). The predictors of persistent oral HPV species 7/9 infections were analyzed using generalized estimating equation models.

Results: The point prevalence of oral HPV varied from 15% to 24% during the 6-year follow-up. Altogether, 18 HPV genotypes were identified either as single or multiple-type oral infections. HPV16 was the most prevalent type at 9.7%–18.4%, followed by HPV18, HPV6, and multiple infections. Altogether, 74 women had persistent oral HPV infection determined as at least two consecutive samples positive with the same HPV genotype. HPV16 and HPV6 were the two most frequent types to persist (76% and 9%) for a mean of 18.6 and 20.2 months, respectively, followed by multiple infections (8%) for 18.3 months. An increased risk for persistent oral HPV infection with species 7/9 was associated with being seropositive for low-risk (LR)-HPV-types at baseline, whereas the use of oral contraceptives and a second pregnancy during follow-up were protective. Clinical oral lesions were detected in 17% of these women, one-third of whom had persistent oral HPV-infections.

Conclusion: HPV16 and HPV6 were the most common genotypes in oral HPV-infections and were also most likely to persist. Use of oral contraceptives and a second pregnancy protected against oral HPV persistence.

Introduction

Emerging evidence points to a causal role for human papillomavirus (HPV) in oral carcinogenesis [1,2]. Natural history of oral HPV infections is poorly understood, and data on the HPV-genotype spectrum in the oral mucosa are scarce. Both low-risk (LR) and high-risk (HR) HPVs have been found in asymptomatic infections as well as in benign and malignant oral lesions [3].

Cross-sectional studies on asymptomatic oral HPV-infection report conflicting results on HPV-DNA prevalence, ranging from 0% to 81% with the mean of approximately 11% [4–11]. HPV16 seems to be the most prevalent genotype, but HPV 12, 18, 53, and 71 have also been reported [7,8,9,12]. Based on a recent meta-analysis on 1,885 cases of oral cavity cancer and 2,248 oral control samples, HPV was found in 33.7% of all cancer samples, compared with only 12% of the control samples [13].

Only a few prospective studies on oral HPV-infections are available. Kurose et al. (2004) found HPV-DNA in 0.6% of oral scrapings from 662 subjects and two of them had a persistent infection over two years [12]. This is much less than recently reported in our family cohort, where 9% of the parents had persistent oral HR-HPV-infection [10]. In another recent study, 15% of 136 HIV-negative individuals had oral HPV-infections, and 60% of these infections persisted for at least six months [14]. The following risk factors of persistent oral HPV-infections have been identified: current smoking, age above 44 years, practicing oral sex, and hand warts [14–15].
The Finnish Family HPV Study was designed to elucidate the dynamics of oral and genital HPV-infections within families [10]. In the present report, point prevalence and persistence of oral HPV-infections are presented at the genotype level during the 6-year follow-up. The predictors of persistent species 7/9 oral HPV infections were also analyzed in univariate and multivariate models. The association of persistent infections with the development of clinical oral lesions was assessed at the study endpoint.

Materials and Methods

Subjects

The Finnish Family HPV Study is a prospective cohort study conducted at the Turku University Hospital and the University of Turku. The study protocol and its amendment (#2/1998 and #2/2006) have been approved by the Research Ethics Committee of Turku University Hospital. Altogether, members of 329 families were enrolled, comprising 329 mothers, 131 fathers and 331 newborns as described in detail previously [10]. The women were originally enrolled in the cohort at 36-weeks (minimum) of their index pregnancy and subsequently followed up (FU) for 6 years. An informed consent in written was obtained from all participants at the first visit. The present analysis is focused on oral HPV-infections among the 324 mothers who had oral swabs available. The mean age of the women was 25.5 years with a range of 18 years to 46 years (median 26.0 years). The flow chart of the present study is shown in Figure 1. Demographic data were collected with structured questionnaires at baseline and during FU (Table 1).

Samples

Oral scrapings for HPV-testing were taken at baseline as well as at 2, 6, 12, 24 and 36-month and 6-year FU-visits. Oral scrapings were taken with a brush (Cytobrush®, MedScan, Malmo, Sweden) from the buccal mucosa of both cheeks as well as the upper and lower vestibular areas. The brush was immersed in 80% ethanol and then immediately frozen and stored at −80°C until use. Cervical samples were collected as described earlier [10,16].

HPV genotyping

HPV-DNA was extracted from the oral scrapings with the high salt method as described previously [17]. Originally HPV-testing for the presence of any HR-HPVs was performed using nested PCR with MY09/MY11 as external and GP05+/GP06+ as internal primers [18]. The PCR products were hybridized with a digoxigenin-labeled HR-HPV-oligoprobe cocktail (HPV-types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56 and 58) to determine whether the samples were HR-HPV-positive (+) or -negative (−) [19].

HPV genotyping was performed with a Multimetrix kit® (Multimetrix, Progen Biotechnik GmbH, Heidelberg, Germany) which detects 24 LR- and HR-HPV-genotypes as follows: LR-HPV6, 11, 42, 43, 44, 44, and 70; and HR-HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. The earlier nested PCR products to detect any high risk HPV was biotinylated by re-amplified with GP05+/bioGP06+-primers. The assay was performed in half of the volume given in the protocol in all steps except the final one. The labeled hybrids were analyzed with a Luminex LX-100 analyzer (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, USA). A median fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cut-off value for each run and HPV-type was 1.5 × background MFI (negative control)+5MFI.

If any sample was positive for HPV16, then the protocol was repeated from the original sample using nested PCR and a bead-based HPV16 genotyping assay [20]. This assay was performed to rule out possible contamination with HPV16 during the previous tests due to several amplifications and the frequency of HPV16 in different samples.

Statistical analysis

All statistical analyses were run using the SPSS® (SPSS, Inc., Chicago, USA) and STATA (Stata Corp., College Station, TX, USA) software packages (PASW Statistics for Windows, version...
### Table 1. Demographic data of the woman at baseline visit.

| Characteristics                  | Number of women (Percentage) |
|----------------------------------|------------------------------|
| **Marital status** (n = 285)     |                              |
| Single                           | 19 (6.7%)                    |
| Living with partner              | 132 (46.3%)                  |
| Married                          | 131 (46.0%)                  |
| Divorced                         | 3 (1.1%)                     |
| **Education** (n = 285)          |                              |
| Compulsory school                | 24 (8.4%)                    |
| Vocational training              | 75 (26.3%)                   |
| Upper secondary school graduate  | 93 (32.6%)                   |
| College graduate                 | 53 (18.6%)                   |
| Academic degree                  | 40 (14.0%)                   |
| **Employment status** (n = 279)  |                              |
| Employed                         | 174 (62.4%)                  |
| Student                          | 43 (15.4%)                   |
| Unemployed                       | 62 (22.2%)                   |
| **Allergic symptoms** (n = 282)  |                              |
| No                               | 156 (55.3%)                  |
| Yes                              | 126 (44.7%)                  |
| **Atopic symptoms** (n = 275)    |                              |
| No                               | 232 (84.4%)                  |
| Yes                              | 43 (15.6%)                   |
| **Menarche** Mean (range) (n = 273) | 12.4 (10–18)              |
| **Abortions** Mean (range) (n = 276) | 0.2 (0–3)                   |
| **Miscarriages** Mean (range) (n = 275) | 0.2 (0–3)                  |
| **Deliveries** Mean (range) (n = 284) | 1.3 (1–4)                  |
| **Age at first sexual intercourse** (n = 285) |              |
| <13 years                        | 7 (2.5%)                     |
| 14–16 years                      | 160 (56.1%)                  |
| 17–19 years                      | 105 (36.8%)                  |
| >20 years                        | 13 (4.6%)                    |
| **Number of lifetime sexual partners** (n = 284) |      |
| 0–2                              | 70 (24.6%)                   |
| 3–5                              | 90 (31.7%)                   |
| 6–10                             | 65 (22.9%)                   |
| >10                              | 59 (20.8%)                   |
| **Number of lifetime sexual partners <20 years of age** (n = 285) |      |
| 0–2                              | 122 (42.8%)                  |
| 3–5                              | 97 (34.0%)                   |
| 6–10                             | 46 (16.1%)                   |
| >10                              | 20 (7.0%)                    |
| **Frequency of sexual intercourse per month** (n = 283) |      |
| 0–1                              | 6 (2.1%)                     |
| 2–4                              | 86 (30.4%)                   |
| 5–10                             | 157 (55.5%)                  |
| >10                              | 34 (12.0%)                   |
| **Practices oral sex** (n = 285) |                              |
| Never                            | 57 (20.0%)                   |
| Occasionally                     | 193 (67.7%)                  |
| Regularly                        | 35 (12.3%)                   |
**Table 1.** Cont.

| Characteristics                                      | Number of women (Percentage) |
|-------------------------------------------------------|-----------------------------|
| **Practices anal sex (n = 285)**                       |                             |
| Never                                                 | 232 (81.4%)                 |
| Occasionally                                          | 51 (17.9%)                  |
| Regularly                                             | 2 (0.7%)                    |
| **Age at onset of oral contraception (n = 284)**       |                             |
| Never                                                 | 22 (7.7%)                   |
| < 13 years                                            | 3 (1.1%)                    |
| 14–16 years                                           | 117 (41.2%)                 |
| 17–19 years                                           | 114 (40.1%)                 |
| > 20 years                                            | 28 (9.9%)                   |
| **Smoking history (n = 284)**                         |                             |
| Never                                                 | 142 (50%)                   |
| Current or past smoker:                               | 142 (50%)                   |
| 1–10 cigarettes/day                                   | 82 (28.9%)                  |
| 11–20 cigarettes/day                                  | 55 (19.4%)                  |
| > 20 cigarettes/day                                   | 5 (1.8%)                    |
| **Use of snuff (n = 259)**                            |                             |
| Never                                                 | 258 (99.6%)                 |
| One can per month                                     | 1 (0.4%)                    |
| **Use of alcohol (n = 284)**                          |                             |
| Never                                                 | 28 (9.9%)                   |
| One dose per day                                      | 1 (0.4%)                    |
| One dose 2–3 times a week                             | 28 (9.9%)                   |
| One dose per week                                     | 89 (31.3%)                  |
| One dose per month                                    | 138 (48.6%)                 |
| **History of sexually transmitted disease (STD) (n = 323)** |                         |
| No                                                    | 261 (80.8%)                 |
| Yes:                                                  | 62 (19.2%)                  |
| Chlamydia trachomatis                                 | 33 (10.2%)                  |
| Genital HSV                                           | 11 (3.4%)                   |
| Multiple STDs                                         | 18 (5.6%)                   |
| **History of genital warts (n = 281)**                 |                             |
| No                                                    | 201 (71.5%)                 |
| Yes                                                   | 80 (28.5%)                  |
| **Age at diagnosis of genital warts (n = 78)**         |                             |
| < 20 years                                            | 36 (46.2%)                  |
| 20–24 years                                           | 31 (39.7%)                  |
| > 25 years                                            | 11 (14.1%)                  |
| **Treatment of genital warts (n = 108)**               |                             |
| No treatment                                          | 41 (38.0%)                  |
| Topical treatment                                     | 31 (28.7%)                  |
| Electrocautery                                        | 4 (3.7%)                    |
| Cryotherapy                                           | 3 (2.8%)                    |
| Laser therapy                                         | 12 (11.1%)                  |
| Surgery                                               | 1 (0.9%)                    |
| Several treatments                                    | 16 (14.8%)                  |
| **History of oral warts (n = 278)**                    |                             |
| Never                                                 | 270 (97.1%)                 |
| Yes, no treatment                                     | 7 (2.5%)                    |
18.0.1 and STATA/SE 11.0) by KS. Frequency tables were analyzed using the \( \chi^2 \)-test with the likelihood ratio or Fisher’s exact test for categorical variables. Differences in the means of continuous variables were analyzed using non-parametric (Mann-Whitney or Kruskal-Wallis) tests for two and more independent samples, respectively.

Outcomes of oral HPV infections and type-specific persistence. At the first level, the genotype-specific outcomes of HPV infection in each woman were assessed by comparing the viral events at each follow-up visit to the baseline HPV status. Genotype-specific persistence was denoted when any case with two or more consecutive follow-up samples were positive for the same individual HPV genotype as a single infection or as a part of multiple-type infection.

Predictors of type-specific persistence in a GEE model. To analyze the predictors of genotype-specific HPV persistence, we determined the predictors of persistence only for the most prevalent HR-HPV-types, i.e., those of species 7 (HPV-types 18, 39, 45, 59, 68, 70 and 85) and species 9 (HPV-types 16, 31, 33, 35, 52, 58 and 67).

A generalized estimating equation (GEE) analysis was used with panel data, clustered by women-ID and run using population-averaged (PA) model [21–22]. The dependent variable was binomial (persistence: yes/no), and hence, the logit link function was used. The independent within-group working correlation structure was the best-fitted covariance pattern, defined by QIC (Quasi-likelihood Information Criterion) [22]. In all models, the robust variance estimator (of 95% CI) was used to account for the

### Table 1. Cont.

| Characteristics | Number of women (Percentage) |
|-----------------|-----------------------------|
| Yes, surgical treatment | 1 (0.3%) |
| **Skin warts (n = 164)** | |
| Hands | 61 (37.2%) |
| Feet | 64 (39.0%) |
| Multiple sites | 39 (23.8%) |

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### Table 2. The genotype-specific point prevalence of oral HPV infection in women followed for 6-years.

| Genotype (% of all samples) | Baseline | 2 mo | 6 mo | 12 mo | 24 mo | 36 mo | 72 mo |
|-----------------------------|---------|------|------|-------|-------|-------|-------|
| HPV+ (any)                  | 55      | 17.0 | 65   | 21.7  | 70    | 24.1  | 54    | 18.8  | 62    | 23.1  | 41    | 15.6  | 27    | 15.1  |
| HPV−                        | 269     | 83.0 | 234  | 78.3  | 220   | 75.9  | 234   | 81.2  | 206   | 76.9  | 222   | 84.4  | 152   | 84.9  |
| HPV6                        | 7       | 2.2  | 0    | 0     | 2     | 0.7   | 2     | 0.7   | 1     | 0.4   | 2     | 0.8   | 0     | 0     |
| HPV11                       | 1       | 0.3  | 0    | 0     | 1     | 0.3   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| HPV16                       | 34      | 10.5 | 55   | 18.4  | 47    | 16.2  | 28    | 9.7   | 37    | 13.8  | 32    | 12.2  | 24    | 13.4  |
| HPV18                       | 1       | 0.3  | 2    | 0.7   | 5     | 1.7   | 2     | 0.7   | 5     | 1.9   | 2     | 0.8   | 0     | 0     |
| HPV31                       | 0       | 0    | 0    | 0     | 1     | 0.3   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| HPV33                       | 0       | 0    | 0    | 0     | 2     | 0.7   | 0     | 0     | 1     | 0.4   | 0     | 0     | 0     | 0     |
| HPV45                       | 0       | 0    | 0    | 0     | 0     | 0     | 0     | 0     | 0.35  | 0     | 0     | 0     | 0     | 0     |
| HPV56                       | 1       | 0.3  | 1    | 0.3   | 0     | 0     | 4     | 1.4   | 0     | 0     | 1     | 0.4   | 0     | 0     |
| HPV58                       | 3       | 0.9  | 1    | 0.3   | 0     | 0     | 7     | 2.4   | 0     | 0     | 0     | 0     | 0     | 0     |
| HPV59                       | 0       | 0    | 0    | 0     | 2     | 0.7   | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| HPV66                       | 3       | 0.9  | 2    | 0.7   | 0     | 0     | 0     | 0     | 3     | 1.1   | 0     | 0     | 0     | 0     |
| HPV70                       | 0       | 0    | 0    | 0     | 2     | 0.7   | 1     | 0.35  | 0     | 0     | 0     | 0     | 0     | 0     |
| **Multiple HPV types (% of all samples)** | 5       | 1.5  | 4    | 1.3   | 8     | 2.7   | 9     | 3.1   | 15    | 5.6   | 4     | 1.5   | 3     | 1.7   |

Distribution of HPV species, genotypes both from single and multiple type infections

| Species 5 (HPV26,51,69,82) | 1 |
| Species 6 (HPV50,53,56,66) | 4 |
| Species 7 (HPV18,39,45,59,68,70,85) | 3 |
| Species 8 (HPV16,31,33,35,52,58,67) | 44 |
| Species 9 (HPV6,11,13,44,55,74) | 10 |

The distribution of oral HPV infections according to the species are also presented. HPV types of the species covered by the Multimetrix® test are bolded.

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Table 3. The duration of the genotype and species specific persistence of oral HPV infection in women.

| HPV  | N  | Mean (months) | Range |
|------|----|---------------|-------|
| HPV6 | 7  | 20.2          | 2.4–75.8 |
| HPV11| 1  | 55.2          |       |
| HPV16| 56 | 18.6          | 2.1–81.2 |
| HPV33| 1  | 11.9          |       |
| HPV58| 1  | 15.2          |       |
| HPV66| 2  | 33.4          | 2.8–63.9 |
| Multiple-type infections | 6 | 18.3 | 4.3–46.5 |
| Species 6 (HPV30,53,56,66) | 2 | 33.4 | 2.8–63.9 |
| Species 9 (HPV16,31,33,35,52,58,67) | 58 | 18.4 | 2.1–81.2 |
| Species 10 (HPV6,11,13,44,55,74) | 8 | 24.6 | 2.4–75.8 |

HPV types covered by the Multimetrix® test are bolded.

Predictors of species-specific persistence

In the univariate GEE-model, being seropositive for LR-HPV at baseline increased the risk of persistence (P = 0.021; OR = 0.49, 95% CI 0.27–0.89 for seronegative women), whereas the second pregnancy during FU (P = 0.001, OR = 0.30, 95% CI 0.15–0.60) decreased the persistence (Table 4). In the multivariate GEE model adjusted for age, these two variables maintained their significance, and an additional predictor was disclosed: the use of oral contraceptives (P = 0.025, OR = 0.29, 95% CI 0.10–0.85), which decreased the risk of persistence.

Discussion

Persistent HR-HPV-infections are the single most important risk factor in cervical carcinogenesis and have been recently implicated in oral and oropharyngeal carcinogenesis as well [1,13,23]. Until now, there have been practically no data on HPV-genotype distributions or their persistence in the oral mucosa. Similarly, the risk factors for the acquisition and persistence of oral HPV-infections are completely unknown. This study is among the first to report HPV-genotype-specific infection in the oral mucosa and is the first to assess the outcome of these infections at the genotype level in a longitudinal setting with 6 years of FU. For the first time, these longitudinal HPV-data are also linked with the development of clinical lesions in the oral mucosa during this observation period.

Before delivery, 17% of the mothers-to-be had HPV-DNA in their oral samples. This prevalence is much higher than that (2.5%) previously reported among pregnant women [24] and also exceeds the average of 11–12% determined from the literature [5–9]. These substantial variations in HPV-detection rates can be
explained by several factors, including the sampling site, sampling method and most importantly HPV-testing techniques as evident from the data presented in Table S1, which is based on three extensive reviews on the literature and 14 recent original articles. Biopsy samples from normal mucosa have resulted in higher HPV detection rate than oral rinse samples. Oral rinse is currently widely used but it is more difficult than scrapings in controlling the quality and quantity of the collected cells. The amount of cells in oral rinse varies depending on the gurgling force and the health of oral mucosa including periodontal health (e.g. grade of inflammation). Furthermore, the origin of the HPV positive cells in rinse would remain unknown. One has to remember that saliva might contain even one to 10 million microbes per one gram which easily lead to the fact that most of the DNA in the rinse sample can even be bacterial DNA, especially when the storage has not been the correct one. Also the use of several primer combinations has increased the HPV detection rate as evident from Table S1. Here we used nested PCR which was reamplified for biotinylation increasing the sensitivity of the test significantly as also found in other studies presented in Table S1. To exclude HPV 16 contamination all HPV16 positive samples were retested using the original DNA and nested PCR. The DNA was extracted with high salt method [6] and thus of good quality. Also the HPV genotyping method was sensitive (Multimetrix assay) and detected 24 different genotypes which is more than in most of the earlier studies. We also collected the samples from non-keratinized oral mucosa to ensure the yield of nucleated cells. Furthermore, the samples were stored in 80% alcohol at 2°C to minimize the oral bacteria load in the sample.

In the present cohort, HPV16 was the single most frequent genotype in oral mucosa at all-time points, followed by multiple-type infections. This finding is in agreement with previous reports [5,12,14]. It was also evident that in the oral mucosa, the HPV-genotype spectrum was similar as encountered in the genital tract.

### Table 4. Predictors of species 7 and 9-specific persistent* oral HPV infections in GEE modeling run in a univariate mode and as adjusted for significant covariates.

| Covariates | Persistent species 7/9 HPV infections |
|------------|---------------------------------------|
|            | Crude | 95% CI | 95% CI | p    | Adjusted | 95% CI | 95% CI | p    |
| Age        | 0.98  | 0.91–1.06 | 0.712  | 0.95  | 0.83–1.08 | 0.476  |
| Mother seroconverted to HR-HPV (yes ref) | 1.14  | 0.59–2.20 | 0.684  |
| Mother seroconverted to LR-HPV (yes ref) | 1.42  | 0.77–2.61 | 0.260  |
| Mother seropositive to HR-HPV at baseline (yes ref) | 0.81  | 0.42–1.55 | 0.528  |
| Mother seropositive to LR-HPV at baseline (yes ref) | 0.49  | 0.27–0.89 | 0.021  | 0.39  | 0.17–0.89 | 0.027  |
| Baseline genital HR-HPV DNA status (+ref) | 1.47  | 0.70–3.07 | 0.302  |
| Baseline PAP smear (<ASCUS ref) | 1.17  | 0.50–2.71 | 0.714  |
| Marital status at baseline (single ref) | 0.90  | 0.60–1.35 | 0.627  |
| Employment status (employed ref) | 1.07  | 0.76–1.51 | 0.679  |
| Age at onset of sexual activity (<13 yrs ref) | 0.83  | 0.52–1.33 | 0.458  |
| No. of sexual partners until age 20 yrs (0–2 ref) | 0.97  | 0.73–1.29 | 0.875  |
| Life-time number of sexual partners | 1.02  | 0.79–1.31 | 0.859  |
| No. of weekly intercourse (no trend) | 0.94  | 0.61–1.49 | 0.803  |
| No. of deliveries in all partnerships | 0.75  | 0.46–1.20 | 0.238  |
| Practices of oral sex (yes ref) | 0.78  | 0.42–1.42 | 0.418  |
| Practices of anal sex (regular ref) | 0.65  | 0.39–1.08 | 0.103  | 0.51  | 0.21–1.26 | 0.148  |
| Initiation of OC usage (<13 yrs ref) | 1.04  | 0.66–1.64 | 0.840  |
| OC use (Y/N) (never use ref) | 0.54  | 0.26–1.15 | 0.112  | 0.29  | 0.10–0.85 | 0.025  |
| Smoking habits (never ref) | 0.62  | 0.34–1.31 | 0.120  |
| Initiation of smoking (10–13 yrs ref) | 0.81  | 0.45–1.48 | 0.509  |
| Consumption of alcohol (no ref) | 2.96  | 0.97–9.13 | 0.059  | 1.44  | 0.25–8.31 | 0.678  |
| History of STD (yes ref) | 1.23  | 0.60–2.54 | 0.561  |
| History of genital warts (yes ref) | 0.64  | 0.35–1.15 | 0.138  | 1.30  | 0.44–3.83 | 0.634  |
| History of oral warts (no history ref) | 1.09  | 0.25–4.71 | 0.907  |
| Second pregnancy during FU visit (no ref) | 0.30  | 0.15–0.60 | 0.001  | 0.42  | 0.19–0.96 | 0.042  |
| Change of marital status during FU | 1.01  | 0.79–1.26 | 0.995  |
| No of current sexual partners (no trend) | 1.09  | 0.30–3.90 | 0.892  |

Species 7 HPV genotypes: 18, 39, 45, 59, 68, 70, and 85. Species 9 HPV genotypes: 16, 31, 33, 35, 52, 58, and 67. *Binary outcome (persistent/not persistent), as defined by persistence of the two original HPV species (same genotype) during the follow-up; Results obtained from GEE with logit link for binary outcomes clustered by woman-ID, 95% CI calculated by robust estimation; @adjusted for age and all significant (and borderline) univariates in the model.

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A small papillomatous lesion in the maxillary labial mucosa. HPV16 and 58 were detected in the oral mucosa at the 1-year follow-up visit. B. Whitish hyperplastic lesion surrounded by mild erythema in the mandibular retromolar area. This woman had a persistent HPV16 infection detected at baseline and again at the 12 and 36-month and 6-year visit. She also had HPV58 at baseline and HPV56 at the 6-year visit. C. Hyperplastic mucosal changes in the tongue. This woman had persistent HPV16 infection at 12, 24 and 36 months. D. Hyperplastic maxillary labial frenulum with persistent HPV16 infection at the 24 and 36-month and 6-year visits. HPV11 was detected at baseline. E. A small papillomatous lesion in the maxillary labial frenulum. This woman had HPV16 at baseline that persisted at the 6-month and 6-year visits. F. Small hyperplastic lesions at the edge of the attached gingiva and oral mucosa. She had persistent HPV16 infection at the 12-month and 6-year visits, the latter accompanied by HPV6.

However, LR-HPV types were more frequent in oral samples than is usually reported in the genital tract. In this same cohort, the most frequent genotypes in the cervix were HPV16, followed by HPV6, 43, 45, 58 and 70 as reported earlier [16]. In the oral mucosa, the following genotypes were found in decreasing order of frequency: HPV16, 6, 18, 56, and 66.

Altogether, six HPV-genotypes were found to persist in 21% of these women. HPV16, followed by multiple-type infection as well as HPV6, were the most frequent genotypes to persist. According to our preliminary 2-year FU-data of this cohort, 9% of the mothers and fathers had persistent oral infection by HR-HPVs [10]. The present data indicate that extending the FU by 4 years revealed substantially more persistent infections. However, the prevalence of HPV persistence is totally depending on the definition of HPV persistence and follow-up time. D’Souza et al. (2007) reported that 60% of oral infections persisted at least 6 months (two visits) in a cohort of 136 women, which seems very high to us as compared with the present long-term longitudinal study [14]. One obvious reason for this finding is the dynamics of HPV-infections: viral clearance takes longer than 6 months, and only a minor proportion of clearance events can be detected within a 6-month FU-period [14].

Oval sex has often been implicated among the risk factors of oral HPV-infections [26–27]. We were unable to confirm this notion in the present study, where no association of sexual habits with persistent oral HPV-infection could be determined. Our cohort is different from many other cohorts as the women here were pregnant at baseline and had a very stable relationship with their spouses during the follow-up. Seropositivity of a woman for LR-HPV at baseline increased the risk of persistent species 7/9 oral infections. The other independent predictor in multivariate GEE analysis for individual genotypes.

Conceived and designed the experiments: SS SG. Performed the experiments: SS JW LW. Analyzed the data: KS SS JR KL JW. Wrote the paper: JR SS KL SG KS JW.

Supporting Information

Table S1 Studies reporting HPV prevalence in normal oral mucosa of healthy individuals since the reviews of Syrjänen and Syrjänen 2000, Kreimer and co-workers (2010) and Syrjänen et al. 2011.

(DOCX)

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