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The clearance of oral high-risk human papillomavirus infection is impaired by long-term persistence of cervical human papillomavirus infection

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Abstract

Persistence of high-risk (HR-) human papillomavirus (HPV) infection of the uterine cervix increases the risk of cervical cancer. Oral HPV infections are among potential covariates of long-term genotype-specific persistent cervical HR-HPV infections. It is not known whether this persistence reflects inability of the host to reject HPV infections in general. A case–control setting was designed to estimate the covariates of long-term persistent cervical HR-HPV infections using multivariate generalized estimating equation (GEE) models. HPV was detected with PCR using GP05+/GP06+-primers and genotyped for 24 HPVs with a Multimetrix-kit. The cases (n = 43) included women who had genotype-specific persistent cervical HR-HPV infection for at least 24 months (24M+) and controls were women who tested repeatedly HPV-negative in their cervical samples (n = 52). These women represent a sub-cohort of the Finnish Family HPV Study. The cases differed significantly from the HPV-negative controls in several aspects: they were younger, had a longer mean time to incident oral HPV infection (40.7 versus 23.6 months), longer duration of oral HPV persistence (38.4 versus 14.1 months), and longer time to clearance of their oral HPV infection (50.0 versus 28.2 months). In multivariate GEE analysis, the second pregnancy during the follow up was the only independent predictor with significant protective effect against 24M+ persistent cervical HR-HPV infections, OR of 0.15 (95% CI 0.07–0.34). To conclude, long-term persistent cervical HR-HPV infections are associated with a prolonged clearance of oral HR-HPV infections while new pregnancy protects against persistent cervical HR-HPV infections.

Keywords: Cervical infections, high-risk human papillomavirus, oral human papillomavirus, persistence, pregnancy, risk factors
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Introduction

Persistent high-risk (HR) human papillomavirus (HPV) infection is necessary for the development of cervical cancer and its precursors [1]. Persistent HPV infections lasting >12 months (12M+) appear to be associated with an increased risk for disease progression [1–3]. Most recent data also imply that the strength of HPV persistence as a predictor of progressive disease varies substantially. It is critically dependent on the reference category used in these assessments, the consistently HPV-negative status as the reference group providing the highest relative risks (RRs) [2]. There is also no generally accepted definition of HPV persistence [1,4]. The most commonly used definition is two or more HPV DNA-positive tests during the follow up [1,5]. Other studies have evaluated persistence based on time to virus clearance or by counting the proportion of HPV-positive visits [6–8]. Consecutive HPV-positivity has been the requirement in most of these studies, but some have also accepted intervening HPV-negative visits as the criteria of persistent HPV [6,8].

Known risk factors for persistent genital HPV infections include older age, smoking, long-term use of oral contraceptives, high parity, number of sexual partners and exposure to other sexually transmitted diseases [9–11]. Currently, the evidence is considered sufficient to confirm the causal association of HPV infection also with oropharyngeal and oral cancers [12]. Most studies on HPV in the head and neck region have focused on HPV prevalence in cancers, whereas the natural history of asymptomatic oral HPV infections is incompletely understood [12,13]. In this study, oral HPV infections are studied among potential covariates of long-term (i.e. 24M+), genotype-specific persistent cervical HR-HPV infections in a case–control setting, with consistently HPV-negative women as the reference group.

Materials and Methods

Subjects

The Finnish Family HPV study (FFHPV) is a prospective cohort study designed to investigate the natural history of oral and genital HPV infections among the members of regular families [4,14]. The study is jointly conducted by the Institute of Dentistry, Faculty of Medicine, University of Turku, and the Department of Obstetrics and Gynaecology, Turku University Hospital (TUH). Altogether, 329 mothers-to-be (mean age 25.5 years) were enrolled at the minimum of 36 weeks of gestation of their index pregnancy and followed-up for 6 years (mean time 54.9 months) after delivery. In the present study, a case–control setting was used to estimate the covariates of long-term persistence of cervical HR-HPV infections. The cases (n = 43) included women who had genotype-specific persistent cervical HR-HPV infection for at least 24 months (24M+) and the controls were women who tested constantly HPV-negative in their repeated cervical samples (n = 52). A structured questionnaire for recording demographic data and potential risk factors was recorded at baseline and 6-year follow-up visits. The Joint Commission on Ethics of Turku University and TUH has approved the study protocol and its amendments (#2/1998 and #2/2006).

Cervical and oral scrapings for HPV genotyping

Cervical and oral scrapings were taken for HPV testing with a cytobrush (Cytobrush, MedScand, Malmö, Sweden) as described earlier [4,14,15]. HPV was detected with PCR using GP05+/GP06+-primers [16]. PCR product was hybridized with a digoxigenin-labelled HR-HPV–oligoprobe cocktail containing 12 HR-HPV oligoprobes [17]. The existing PCR products were biotinylated by re-amplification with GP05+ and bio-GP06+ primers for HPV genotyping by Luminex-based Multimetrix kit (Progen Biotechnik GmbH, Heidelberg, Germany), which detects 24 low-risk (LR-) and HR-HPV genotypes (LR-HPV: 6, 11, 42, 43, 44, 70; HR-HPV: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) [18].

Blood samples for HPV serology

Blood samples for analysis of HPV antibodies were taken at baseline and at 12, 24 and 36 months of follow up. Antibodies to the major capsid protein L1 of HPV types 6, 11, 16, 18 and 45 were analysed in the German Cancer Research Centre (Heidelberg), with Multiplex HPV serology [19]. The cut-off value of 200 median fluorescence intensity was used [20].

Statistical analyses

To analyse the potential covariates of long-term (24M+) genotype-specific persistent cervical HR-HPV infections in a case–control setting, a sub-cohort of FFHPV study was built up: (i) the case group which included 43 mothers who had a genotype-specific persistent cervical HR-HPV infection of at least 24M+ duration, and (ii) the control group comprising 52 mothers who tested HPV-negative in their cervical samples throughout the follow-up period. Key epidemiological characteristics were compared between these two groups. Oral HPV persistence was defined as two or more consecutive oral HPV-positive visits during the follow up. Frequency tables were analysed using the χ^2-test, with the likelihood ratio or Fisher's exact test for categorical variables. Differences in the means of continuous variables were analysed using non-parametric (Mann–Whitney or Kruskal–Wallis) tests for two and multiple independent samples, respectively.

Generalized estimating equation (GEE) modelling was used to analyse the predictors of long-term persistence of cervical HR-HPV infections. In univariate GEE models, we first tested all the covariates recorded at baseline (including serological data) and previously implicated as potential risk factors of HPV infections in this cohort [4,14]. In the final multivariate GEE model, only the variables that were statistically significant in the univariate model were entered, adjusted for age (continuous variable). All statistical analyses were run using SPSS® (IBM Corp., Armonk, NY, USA) and STATA (Stata Corp., College Station, TX, USA) software packages (PASW Statistics for Windows 19.0.1 and STATA/SE 12.0).

Results

The key characteristics of the cases and the controls are shown in Table 1. The mean follow-up time for the cases was 65.2 months (95% CI 58.5–71.9) and that of the controls was 38.4 months (95% CI 30.2–46.7). Among the 43 cases, the

TABLE 1. Key epidemiological characteristics of the cases and controls

Characteristics	Cases persistent cervical HPV infection (*n* = 43)	Controls constantly HPV-negative in cervical samples (*n* = 52)	p value*
Mean age (years, 95% CI)	**25.2 (24.4–25.9)**	**26.4 (25.4–27.3)**	**0.054**
Not married (single)	2.4% (1/42)	0% (0/51)	0.268
Employment			
Employed	59.5% (25/42)	70.6% (36/51)	0.534
Student	16.7% (7/42)	11.8% (6/51)	
Unemployed	23.8% (10/42)	17.6% (9/51)	
Age at first intercourse			
≤13 years	2.4% (1/42)	2.0% (1/51)	0.938
14–16 years	57.1% (24/42)	51.0% (26/51)	
17–19 year	35.7% (15/42)	41.2% (21/51)	
>20 years	4.8% (2/42)	5.9% (3/51)	
Number of sex partners before age 20 years			
0–2 partners	50.0% (21/42)	43.1% (22/51)	0.869
3–5 partners	26.2% (11/42)	29.4% (15/51)	
6–10 partners	14.3% (6/42)	19.6% (10/51)	
>10 partners	9.5% (4/42)	7.8% (4/51)	
Number of lifetime sex partners			
0–2	35.7% (15/42)	21.6% (11/51)	0.449
3–5	21.4% (9/42)	29.4% (15/51)	
6–10	28.6% (12/42)	29.4% (15/51)	
>10	14.3% (6/42)	19.6% (10/51)	
Number of intercourses/month			
0–1	2.4% (1/42)	2.0%1/51)	0.190
2–4	35.7% (15/42)	19.6% (10/51)	
5–10	52.4% (22/42)	60.8% (31/51)	
>10	7.1% (3/42)	17.6% (9/51)	
Having oral sex (yes)	83.3% (35/42)	76.5% (39/51)	0.451
Having anal sex (yes)	23.8% (10/42)	15.7% (8/51)	0.430
Age at starting oral contraception			
Never	9.5% (4/42)	6.0% (3/50)	0.616
14–16 years	31.0% (13/42)	44.0% (22/50)	
17–19 years	45.2% (19/42)	38.0% (19/50)	
>20 years	14.3% (6/42)	12.0% (6/50)	
Use of oral contraception (yes)	90.5% (38/42)	94.0% (47/50)	0.698
Ever been smoker	48.8% (20/41)	56.0% (28/50)	0.532
Age of initiating smoking			
10–13 years	10.5% (2/19)	20.0% (4/20)	0.931
14–17 years	73.7% (14/19)	65.0% (13/20)	
18–21 years	10.5% (2/19)	10.0% (2/20)	
22–25 years	5.3% (1/19)	5.0% (1/20)	
Ever had a sexually transmitted disease	76.7% (33/43)	73.1% (38/52)	0.813
Never had genital warts	66.7% (28/42)	64.0% (32/50)	0.829
Never had oral papillomas	92.7%(38/41)	95.9% (47/49)	0.656
Seropositive to HPV 16, 18, 45 (Baseline)	42.9% (18/42)	26.9% (14/52)	0.128
Seropositive to HPV 6 or 11 (Baseline)	50.0% (21/42)	65.4% (34/52)	0.146
Oral HR-HPV positive (Baseline)	14.0% (6/43)	13.5% (7/52)	1.000
Seroconversion to HPV 16, 18, or 45	27.9% (12/43)	21.2% (11/52)	0.479
Seroconversion to HPV 6 or 11	25.6% (11/43)	25.0% (13/52)	1.000
Pap smear ≥ASCUS	16.7% (7/42)	15.4% (8/52)	1.000
Persistent oral HPV	30.2% (13/43)	21.6% (11/51)	0.354
Mean time to incident oral HPV infection (Oral baseline negative) (months)	**40.7 (30.8-50.6)**	**23.6 (16.5-30.6)**	**0.005**
Mean time of oral persistent HPV infection (months)	**30.6 (15.6-45.6) (*n* = 13)**	**11.7 (7.6-15.9) (*n* = 11)**	**0.023**
Mean time to oral HPV clearance (all at risk) (months)	**50.0 (36.4-63.6) (*n* = 24)**	**28.2 (18.4-38.0) (*n* = 28)**	**0.009**

*Significant associations are highlighted in bold (p ≤0.05), Significance between Cases and Controls; analysis of variance test for all continuous variables; Fisher's exact test for all comparisons where any cell in the contingency table contains fewer than five observations.
ASCUS, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus.

following HPV genotypes were recorded to persist in the cervix for 24M+: HPV16 (*n* = 36), HPV35 (*n* = 1), HPV45 (*n* = 1), HPV52 (*n* = 2) and multiple HPV types (*n* = 3) (data not shown). The mean time of cervical HPV persistence was 36.0 months (95% CI 24.1–78.8). The cases were younger (mean age 25.2 versus 26.4 years), had longer (mean) time to incident oral HPV infections (40.7 versus 23.6 months), longer duration of persistent oral HPV infections (30.6 versus 11.7 months), and the clearance of their oral HPV infections was more prolonged (50.0 versus 28.2 months).

The persistent oral HPV infections among the cases included the following HPV types: HPV16 (*n* = 13) and multiple HPV types (*n* = 5). Type-specific concordance between the persisting cervical and oral HPV (*n* = 13) was not significant. However, as to the overall HPV persistence by follow-up visit, oral and cervical HPV persistence were significantly correlated (Likelihood ratio, 11.8; p 0.001). Women with a persistent cervical HPV infection had an increased risk of having a co-incident persistent oral HPV infection at the same visit, with OR of 3.31 (95% CI 1.71–6.39).

As to the dynamics of genotype-specific HPV serology (for HPV6, 11, 16, 18 and 45) in the cases and the controls, no differences were disclosed in HPV antibody titres at any of the follow-up visits. The same was true with the rates and times of type-specific sero-conversion. However, the cases were more

TABLE 2. Covariates of long term (24M+ months) persistent[a] cervical high-risk human papillomavirus infections in univariate and multivariate generalized estimating equation models[b]

Covariates	Univariate GEE model		Multivariate GEE model	
	OR	95% CI	OR[c]	95% CI
Age	**0.90**	**0.82–0.99**	0.89	0.78–1.01
Mother seroconverted to HR-HPV (yes; ref.)	0.73	0.37–1.44		
Mother seropositive to HR-HPV at baseline (yes; ref.)	1.01	0.56–1.80		
Baseline oral HR-HPV DNA status (neg; ref.)	1.23	0.70–2.17		
Baseline PAP smear (<ASCUS; ref.)	0.57	0.21–1.52		
Living with partner at baseline (single; ref.)	0.86	0.13–5.67		
Employment status (employed; ref.)	1.15	0.81–1.63		
Age at onset of sexual activity (<13 years; ref.)	0.78	0.47–1.31		
No. of sexual partners until age 20 years (0–2; ref.)	1.07	0.77–1.49		
Life-time number of sexual partners (0–2; ref.)	0.94	0.70–1.25		
No of weekly intercourse (0–1; ref.)	0.77	0.51–1.17		
No of deliveries in all partnerships (1; ref.)	0.52	0.23–1.17		
Practices of oral sex (no; ref.)	1.45	0.64–3.29		
Practices of anal sex (no; ref.)	1.52	0.75–3.09		
Initiation of OC usage (<13 years; ref.)	1.21	0.79–1.86		
OC use (Y/N) (never use; ref.)	0.62	0.21–1.78		
Smoking habits (never; ref.)	1.22	0.66–2.27		
Initiation of smoking (10–13 years; ref.)	1.05	0.55–1.99		
History of sexually transmitted disease (no; ref.	0.96	0.47–1.98		
History of genital warts (yes; ref)	1.02	0.53–1.96		
History of oral warts (no history; ref.)	1.28	0.44–3.75		
Second pregnancy during follow up (no; ref.)	**0.16**	**0.08–0.31**	**0.15**	**0.07–0.34**
Change in marital status during follow up (no; ref.)	1.05	0.84–1.31		
New partner during follow up (no; ref.)	**1.90**	**1.01–3.58**	1.49	0.86–3.26
No of current sexual partners (0; ref.)	0.70	0.26–1.89		

[a]Binary outcome (persistent/not persistent), as defined by persistence at least 24M+ during the follow up.
[b]Results obtained from GEE with logit link for binary outcomes, clustered by woman's ID, 95% CI calculated by robust variance estimation.
[c]Adjusted for age and all significant univariates in the model; significant results are shown in bold type.
ASCUS, atypical squamous cells of undetermined significance; GEE, generalized estimating equation; HR-HPV, high-risk human papillomavirus; OC, oral contraceptive.

likely to be HPV16-seropositive at the baseline visit than the controls (40.5% versus 19.2%; OR 2.1, 95% CI 1.08–4.10).

Table 2 shows the covariates associated with 24M+ persistence of cervical HR-HPV infections. In the univariate GEE model, three significant predictors were found: (i) age; the risk was reduced with age, OR of 0.90 (95% CI 0.82–0.99), (ii) second pregnancy during the follow up; women with a second pregnancy were protected (OR 0.16, 95% CI 0.08–0.31); and (iii) having a new partner during the follow up, increasing the risk (OR 1.90, 95% CI 1.01–3.58). After adjustment for age in the multivariate GEE model, only the second pregnancy retained its significance as an independent predictor of 24M+ persistence. Women who had a second pregnancy during the follow up were significantly protected against persistent HR-HPV infection (OR 0.15, 95% CI 0.07–0.34).

Discussion

According to our knowledge, this is one of the first studies to demonstrate in a longitudinal setting that long-term persistence of cervical HR-HPV infections might affect the outcome of HPV infections at other mucosal sites, i.e. oral mucosa in this case. Our data implicate that women with persistent cervical HPV infections of 24M+, acquired an incident oral HPV infection later (40.7 versus 23.6 months), their oral HR-HPV infections persisted nearly three times longer than in (cervical) HPV-negative controls (30.6 versus 11.7 months), and also cleared more slowly than among women with no cervical HPV infection (50.0 versus 28.2 months). Also the risk for developing a persistent oral infection was over three-fold higher among the cases than in the controls (OR 3.31).

Recent cross-sectional studies have shown that the prevalence of oral HPV infections is higher among women with concurrent cervical HPV infection [13,21]. One of these studies also reported a moderate type-specific HPV concordance between the oral and genital HPV types [21]. We could not record any significant concordance between the persistent cervical and oral HPV genotypes, which is most likely because of the limited number of such cases and possibly because of the longitudinal setting of the present study. It has been speculated that subjects with a concomitant cervical and oral HPV infection might represent a subgroup of women with an increased inherent susceptibility to HPV infection, possibly due to an impaired immune response [13]. Our serological data from the present case–control setting showed that women in the case group were more likely to be HPV16-seropositive at their baseline visit. They also had lower titres of HPV16 antibodies at all follow-up visits compared with the women in the HPV-negative control group. Although these differences did not reach statistical significance, these data might indicate a tendency towards compromised antibody response among the

cases and their failure to clear chronic cervical HPV infections, particularly those caused by the HPV16 genotype. Our previous findings in the whole FFHPV (women) cohort disclosed no correlations between oral HPV DNA detection and HPV serology [22]. Interestingly, however, the data did show the highest titres of HPV type-specific antibodies among women who cleared their cervical HPV16 infection, whereas the women who acquired incident cervical HPV16 infections had the lowest HPV16 antibody levels [22].

In the present study, women with 24M+ HPV-persistence were 1 year younger than the HPV-negative controls (25.2 versus 26.4 years). Consequently, in the GEE model, the risk of persistence declined significantly with age. Because all the women in the FFHPV study comprise a rather homogeneous cohort, i.e. all were young mothers-to-be at enrolment [14], these data indicate that there may be a subgroup of women in whom HPV persistence is established at a young age and not later, as suggested by some previous studies [9–11]. Only one recent study showed that younger age can be a risk factor for persistent HPV infections [23]. Interestingly, our recent results showed that children who were born to the mothers with incident cervical intraepithelial neoplasia lesions already had HPV16-specific cell-mediated immunity [24]. All of these children were sexually naive and were not vaccinated against HPV. Accordingly, HPV infections at an early age might affect the outcome of genital or oral HPV infections later in life. The present observations fit with the dynamic model of cervical HPV infections, peaking at around 24 years of age, with steadily declining age-specific HPV prevalence until menopause [3,25].

In estimating the covariates of 24M+ persistent cervical HR-HPV infections, only the second pregnancy during the follow up remained significant in the adjusted GEE model. This is in line with our previous results from the whole FFHPV cohort, showing that the second pregnancy was a potent protective factor against incident cervical HPV infections [26]. Similarly, we have demonstrated that the risk for incident oral HPV infections and their persistence is markedly decreased by a new pregnancy [15,27]. Most previous studies have focused only on differences in HPV prevalence among the pregnancy trimesters [28,29]. A recent study showed that among persistent HPV infections, childbirth proved to be a cofactor for high-grade cervical disease but not pregnancy [30]. HPV and pregnancy is still a controversial issue, and the mechanistic explanation for this observed protective effect in our study must be complex. We have previously found that women committed to the second child in our FFHPV cohort did not have most of the known life-style behavioural risk factors of HPV infection [31]. In addition to these obvious differences in the behavioural pattern between the women with and without a second pregnancy, we cannot ignore the effects of pregnancy-associated hormonal changes as potential specific modulators of the host response to HPV.

The strength of the present study is the case–control setting; the cases comprise women with long-term persistent cervical HR-HPV infections, and the controls consist of women who remained completely HPV-negative throughout their follow up. Noteworthy, our definition of HPV persistence was exceptionally stringent compared with many previous studies, because a minimum of 24 months of persistence was required, recorded at consequent follow-up visits [1,2]. Furthermore, our study is the first to correlate oral and cervical HPV infections in a longitudinal setting, in contrast to the previous cross-sectional studies [13,21].

Taken together, these results strongly suggest that long-term cervical HR-HPV persistence might be an indicator of a compromised host response to co-existent oral HPV infections as well. Becoming pregnant during the follow up seems to confer a significant protective effect against long-term persistence of cervical HR-HPV.

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Transparency Declaration

The authors declare no conflicts of interest.

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