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