Genotype-Specific Clearance of Genital Human Papillomavirus (HPV) Infections among Mothers in the Finnish Family HPV Study

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The majority of cervical human papillomavirus (HPV) infections in young women are transient, but whether the clearance differs among different HPV genotypes and the different factors predicting genotype-specific clearance are partly unknown. In the Finnish Family HPV Study, 131 of 252 women (mean age, 25.5 years) cleared their infection during the prospective follow-up of 6 years (median, 62.4 months; range, 1.6 to 94.5 months). Cervical scrapings collected at each visit were tested for 24 low-risk and high-risk (HR) HPV types with multiplex HPV genotyping. Poisson regression (panel data) was used to estimate predictors for the clearance of species 7/9 HPV genotypes. Of all HPV genotypes detected in these women, multiple-type and HPV type 16 (HPV16) infections showed clearance least frequently (46.1% and 50.5%, respectively). The actuarial and crude mean times to first clearance were variable among different genotypes. The actuarial clearance rate (events/person-time at risk) was highest for HPV16 and multiple-type infections, while HPV66 and -82 had the highest crude clearance rate. Independent predictors increasing type-specific clearance of species 7/9 HPV genotypes were older age (incidence rate ratio [IRR] = 1.1; 95% confidence interval [95% CI], 1.03 to 1.18; \( P = 0.002 \)) and baseline oral HR HPV DNA-negative status (IRR = 2.94; 95% CI, 1.03 to 8.36; \( P = 0.042 \)), while a higher number of sexual partners during the follow-up decreased the probability of clearance (IRR = 0.35; 95% CI, 0.15 to 0.83; \( P = 0.018 \)). To conclude, HPV16 and multiple-type infections showed the lowest clearance among young mothers. Increasing age and negative oral HR HPV DNA status at baseline were associated with increased clearance, whereas a higher number of current sexual partners decreased the probability of species 7/9 HPV genotype clearance.

Genital human papillomavirus (HPV) infections are transient in most cases (3, 7). Most studies on HPV clearance have addressed high-risk (HR) HPV types collectively and/or have compared clearance between HR and low-risk (LR) HPV types (3, 4, 12, 13, 17, 18, 21, 23, 24). Earlier data suggest that HR HPV infections usually clear more slowly than LR HPV infections (4, 25) and that the likelihood of an infection not clearing increases in parallel with its duration (7, 13).

It was not until recently that data on HPV clearance at the genotype level were available (5, 9, 19, 25). The results indicate that infection with HPV type 16 (HPV16) has the lowest tendency for clearance. Accurate data on actual and crude clearance times and clearance rates (CRs) for individual genotypes are needed to understand the natural history of HPV infections.

The present study is one of the first to assess the frequency of HPV type-specific clearance as well as the actuarial and crude clearance times and clearance rates for the 24 most common LR and HR HPV genotypes. The study was performed among newly delivered mothers who were followed up for 6 years in the Finnish Family HPV Study. In addition, predictors of species 7/9 HPV genotype clearance were analyzed in a panel Poisson regression model.

MATERIALS AND METHODS

Subjects. The Finnish Family HPV Study is a prospective cohort study conducted jointly by the Department of Obstetrics and Gynecology, Turku University Hospital (TUH), and the Institute of Dentistry, Faculty of Medicine, University of Turku. The subjects in this cohort are pregnant women who were recruited at a minimum of 36 weeks of pregnancy (baseline) (16) and followed up for 6 years (mean, 54.9 months; standard deviation [SD], 27.3 months; median, 62.4 months; range, 1.6 to 94.5 months) after delivery (10). The Joint Commission on Ethics of Turku University and TUH has approved the study protocol and its amendments (no. 2/1998 and 2/2006). Altogether, 329 mothers were enrolled in the cohort (mean age, 25.3 years), of whom 252 tested HPV positive at least once during the follow-up (FU) and were eligible for this analysis. For 20 women unable to participate in the FU, the women were excluded from this study, remained HPV negative throughout the FU period, while the remaining 31 women were sampled only once during the FU. The women in this study are of Caucasian origin, have the same ethnic background, and are representative of the Finnish population. The flowchart for the study setting is described in Fig. 1. Some of the women were lost to FU, mainly due to difficulties in attending or to family reasons. A structured questionnaire for recording demographic data and potential risk factors was recorded at baseline and repeated at 36-month and 6-year FU visits. Selected data from these records were used for risk assessment in the present Poisson regression analysis.

Samples. The cervical scrapings for HPV testing were taken at the baseline and at 3-, 12-, 24-, and 36-month and 6-year visits (counted from the study entry). Sampling was done with a cytobrush (MedScand, Malmo, Sweden) from the uterine cervix using a sampling medium of 0.05 M phosphate-buffered saline with 100 μg gentamicin. The samples were immediately frozen at −20°C and stored at −70°C. The scrapings were collected as described earlier (15), and only the baseline DNA data were used in the statistical analyses.

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Pap smears. A routine Pap smear was taken from all women at baseline and at 12, 24, and 36 months and 6 years by using the conventional three-sample technique with a wooden spatula and cytobrush (MedScand, Malmö, Sweden) as described earlier (16).

HPV genotyping. The HPV genotyping was done with a multiplex HPV genotyping kit (Multimetrix; Progen Biotecnich GmbH, Heidelberg, Germany) as outlined in Fig. 2. The kit identifies the following 24 LR and HR HPV genotypes: HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82 (20). With a Luminex LX-100 analyzer (Bio-Plex 200 system; Bio-Rad Laboratories, Hercules, CA), the medium fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cutoff value for each run and HPV type was 1.5 times the background MFI (negative control) plus 5 MFI units.

HPV genotyping was done using the earlier PCR product, which was now reamplified with the GP05/H11001 and bio-GP06/H1005 primers (22). DNA originally was extracted from scrapings by the high-salt method (11). The PCR products were then hybridized with a digoxigenin-labeled HR HPV oligoprobe cocktail (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82) (20). With these options, Poisson regression for panel data is similar to the PA generalized estimating equation (GEE) model, with the two giving identical results.

In univariate Poisson analysis, we first tested all covariates recorded in the baseline questionnaire as well as some selected variables from the FU questionnaire (e.g., a record of new partners) that were previously implicated as potential HPV risk factors in this cohort (16). In the final multivariate model, only variables that were significant (or borderline significant) in univariate correlation was the best-fit covariance pattern, defined by the quasilikelihood information criterion (QIC) (6). With these options, Poisson regression for panel data is similar to the PA generalized estimating equation (GEE) model, with the giving identical results.

RESULTS

Outcomes of HPV infection and type-specific HPV clearance. The outcomes of HPV infection are presented in Fig. 3. The following six different outcome patterns were first defined: (i) always negative, (ii) incident HPV (women who were HPV negative at baseline and acquired incident HPV infection during the FU), (iii) type-specific persistence, (iv) non-type-specific persistence, (v) fluctuation, and (vi) clearance (transient). In this study, we focused on those 252 women who tested HPV positive at least once during the FU and thus accumulated time at risk for HPV clearance. Of these 252 women, 131 cleared their infection during FU. Clearance was defined as when a woman with a previous HPV-positive test had a negative test (at any FU visit) and remained HPV negative until the end the last visit. The outcome for those women who had another positive HPV test following a negative result was classified as fluctuation and was excluded from this analysis.

Type-specific clearance of HPV infections. Of the 329 women enrolled in this study, 252 tested HPV positive at some point, and of those, 131 experienced a clearance event during the FU. The mean follow-up time for these 252 women was 58.8 ± 25.1 (SD) months (median, 65.0; range, 6 to 95). By the end of FU, 100% of HPV11, -43, and -51 infections cleared, as did 83.3% (46/55) of the cases.

The interval times (months) to the first clearance event were calculated from the first HPV-positive visit (baseline or any FU visit) to the first clearance event, separately for both actuarial and crude times. To calculate the actuarial clearance time,
both women who had a clearance event and those who did not (n = 252) were included in Kaplan-Meier analysis. The longest actuarial times to clearance were found for single cases of HPV33 and HPV44, which did not show clearance (Table 1). This was followed by HPV66, -35, and -31, with actuarial times of 48.1, 45.6, and 45.5 months, respectively. On the other hand, the shortest actuarial times were recorded for HPV73, -11, and -59, i.e., 11.8, 12.4, and 12.8 months, respectively. Most of the remaining types (HPV16, -18, -52, -58, and -70) had actuarial times ranging between 30.7 to 36.9 months, whereas multiple-type infections had a somewhat longer clearance time of 41.1 months. To calculate the crude clearance times, only those 131 women with a clearance event were included, and these are therefore shorter than the actuarial times. The longest crude time of 49.5 months was recorded for HPV52. This was followed by HPV16, -31, -43, -51, -56, and -70, with crude times of between 22.1 and 28.5 months. The shortest crude time of 10.4 months was shown by HPV73, which was somewhat shorter than that for the remaining genotypes, ranging between 11.9 and 14.8 months. For multiple-type infections, the crude time to clearance was 21.7 months.

Of the individual HPV species, species 8 was cleared in 100% of the infection (n = 2), with both actuarial and crude times to clearance being 24.8 months. This was followed by HPV66, -35, and -31, with actuarial times of 48.1, 45.6, and 45.5 months, respectively. On the other hand, the shortest actuarial times were recorded for HPV73, -11, and -59, i.e., 11.8, 12.4, and 12.8 months, respectively. Most of the remaining types (HPV16, -18, -52, -58, and -70) had actuarial times ranging between 30.7 to 36.9 months, whereas multiple-type infections had a somewhat longer clearance time of 41.1 months. To calculate the crude clearance times, only those 131 women with a clearance event were included, and these are therefore shorter than the actuarial times. The longest crude time of 49.5 months was recorded for HPV52. This was followed by HPV16, -31, -43, -51, -56, and -70, with crude times of between 22.1 and 28.5 months. The shortest crude time of 10.4 months was shown by HPV73, which was somewhat shorter than that for the remaining genotypes, ranging between 11.9 and 14.8 months. For multiple-type infections, the crude time to clearance was 21.7 months.

Of the individual HPV species, species 8 was cleared in 100% of the infection (n = 2), with both actuarial and crude times to clearance being 24.8 months. Species 5, 10, and 7 showed clearance in 80% (n = 4/5), 77.8% (n = 7/9), and 67.9% (n = 19/28) of the cases, respectively. For species 9,
clearance was only 49.2% (n = 61/124), which is similar to those for the rare species 6 and 11, i.e., 40% (n = 2/5) and 50% (n = 1/2), respectively. The actuarial times to clearance were longest for species 6 (46.2 months), followed by species 9, with a median time of 37.4 months (Table 1). Both the actuarial and crude clearance times were shortest for species 11, being only 11.7 and 10.4 months, respectively. The longest crude times to clearance were recorded for species 9 and 8, i.e., 37.4 and 24.3 months, respectively.

The cumulative clearance of species 7 and 9 was compared with that of species 10 in univariate survival (Kaplan-Meier analysis (Fig. 4). Species 10 (benign types) showed a significantly more rapid and complete clearance than species 7 and 9, which also markedly deviated from each other, with species 9 showing the least cumulative clearance (log rank test, P = 0.013). When HPV16 alone was compared with all other genotypes in a similar Kaplan-Meier analysis, the difference was also significant (P = 0.015), with HPV16 clearance being almost 20% less than that of all other genotypes (data not shown).

CRs. Both actuarial and crude clearance rates (CRs) were also calculated and were expressed as events per 1,000 woman-months at risk (WMR). To obtain genotype-specific actuarial clearance rates, the number of clearance events for each indi-
individual genotype and species was divided by the total WMR (i.e., 9.146 months accumulated by all 252 HPV-positive women), thus also including the women with no clearance event. Of the single genotypes, the most frequent genotype, HPV16, also showed by far the highest CR, 5.9/1,000 WMR (95% CI, 4.3 to 7.4), followed by multiple-type combinations, which cleared at a rate of 3.8/1,000 WMR (95% CI, 2.5 to 5.1). The actuarial CR for HPV18 was markedly lower, at 0.65/1,000 WMR (95% CI, 0.1 to 1.2). Due to the dominant role of HPV16, species 9 showed the highest CR of 6.7/1,000 WMR (95% CI, 5.0 to 8.3), far exceeding that (2.0/1,000 WMR) of species 7 (Table 1). This is logical, since the CR is likely to correlate with the rate of detection of a specific HPV type.

To calculate crude clearance rates, only the women with clearance events were included and the number of clearance events for each individual genotype (or species) was divided by the WMR accumulated by those women only and therefore is a robust measure to compare different HPV genotypes and HPV species. Of all genotypes, HPV66 and -82 cleared most rapidly, both accumulating clearance events at a rate of 83.2/1,000 WMR (95% CI, 5.0 to 8.3), far exceeding that (2.0/1,000 WMR) of species 7 (Table 1). This is logical, since the CR is likely to correlate with the rate of detection of a specific HPV type.

When all significant and borderline significant variables of univariate Poisson analysis were entered in the multivariate Poisson model together with patient age, three variables retained their significance as independent predictors of species 7/9 clearance: (i) age (clearance was more common with increasing age [P = 0.002]), (ii) baseline oral HR HPV DNA status (being HR HPV negative increased the probability of clearance) (incidence rate ratio [IRR] = 2.94; P = 0.042), and (iii) number of sexual partners during FU (all women with ≥2 partners failed to clear) (IRR = 0.35; 95% CI, 0.15 to 0.83; P = 0.018).

**DISCUSSION**

The present study is the first to provide detailed information on both actuarial and crude clearance times and clearance rates at the HPV genotype and species levels. Not unexpectedly, the lowest clearance frequency was recorded for HPV16 and multiple-type infections, of which only 51.6% and 50.5% cleared, respectively. In Kaplan-Meier analysis, HPV16 clearance was almost 20% less than that of all other genotypes. In other studies, 80.7%, 69%, and 51.9% of HR HPV infections cleared at between 14 and 19 months of FU (5, 18, 21). Of LR HPV types, 81% were shown to clear within 12 months FU (5), and the majority of type-specific clearance occurred in 2 years (14). When stratified by HPV species, species 10 (benign types) shows a significantly more rapid clearance than species 7 and 9. This is in alignment with the results indicating that species 9 has the lowest clearance rate and the longest disease duration (5, 25).

While also including women with no clearance, the actuarial times indicate how long it takes among HPV-positive women to clear the infection by a specific genotype. To the best of our knowledge, actuarial clearance of different HPV types has not been previously reported. The crude times for each genotype indicate the time required for clearance in women who experience such an event, providing a robust measure to compare different HPV genotypes (Table 1). In the present series, our data on HPV16 are remarkably similar to recently reported data showing mean clearance times of between 17.1 and 22 months during 19 months and 48 months of FU time, respectively (8, 18). For HPV6 and -11, our crude times were 14.8 and 12.4 months, respectively, which are also very similar to those reported previously (9.3 and 8.4 months, respectively [8], as well as 9.5 months for HPV6/11 [25]). These data confirm that the crude clearance times for HR HPV types are almost twice as long as those for LR HPV (22.1 to 28.1 months versus 10.4 to 14.8 months).

The actuarial and crude CRs are markedly different because of the significantly different denominators. Comparison between individual studies is difficult, particularly when FU times and cohort sizes are substantially different. In our cohort, HPV16 showed a markedly lower crude CR of 45.2/1,000 WMR than in a recent study reporting a crude CR of 72.0/1,000 WMR for HPV16 during 15 months of FU (5). When analyzed by species, CRs of 44.9/1,000 WMR and 59.3/1,000 WMR were recorded for species 9 and 7, respectively. These are somewhat lower than those previously reported for species 9 (143.1/1,000 WMR and 76.5/1,000 WMR) and for species 7 (110.7/1,000 WMR and 94.1/1,000 WMR) (5, 25). These dif-
The second significant predictor of clearance was the number of current sexual partners, recorded at the 36-month mid-
point during FU. Accordingly, those women who reported no partner during the FU all cleared their infection, in contrast to those who had two or more, none of whom cleared. Previous studies have not found an association between the number of current sexual partners and clearance (19, 25). This observation is completely new and has not even been assessed in any previous studies due to the lack of oral samples. This issue will be explored in detail during analysis of the genotype-specific oral HPV data from the Finnish Family HPV study in due course. Interestingly, of the 12 women who reported a history of oral warts, none cleared their genital HPV infections, which is consonant with the observation that baseline oral HR HPV DNA-positive status reduced the likelihood of clearance.

To conclude, HPV16 and multiple-type infections showed the lowest clearance among newly delivered mothers. This is the first study where both (i) actuarial and crude times to the first clearance event and (ii) actuarial and crude CRs were evaluated at the genotype and HPV species levels. The significant independent predictors of species 7/9 clearance include (i) age, (ii) having >2 current sexual partners, and (iii) baseline oral HR HPV DNA-
negative status increase the clearance, while having multiple current sex partners decreases the probability of clearing species 7/9 infections.

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