High-Risk Types of Human Papillomavirus (HPV) DNA in Oral and Genital Mucosa of Infants during Their First 3 Years of Life: Experience from the Finnish HPV Family Study

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Background. This study is aimed to clarify data on the acquisition, persistence, and clearance of high-risk types of human papillomavirus (HPV) DNA from the mucosa and the determinants of persistent mucosal HPV infection in infants.

Methods. Oral and genital scrapings from 324 infants were collected at birth, 3 days after delivery, and 1, 2, 6, 12, 24, and 36 months after delivery and tested for the presence of HPV DNA by nested polymerase chain reaction and hybridization with 12 high-risk HPV oligoprobes. HPV status and demographic data for parents were analyzed.

Results. During the follow-up period (median duration, 26.2 months), HPV DNA was found to be present in 12%–21% of oral scrape samples and in 4%–15% of genital scrape samples obtained from the infants. Oral HPV infection was acquired by 42% of children, cleared by 11%, and persisted in 10% of the infants, whereas 37% were never infected. The corresponding figures for genital HPV infection were 36%, 14%, 1.5%, and 47%. Kaplan-Meier analysis revealed that both the cumulative incidence of infection and clearance of HPV were parallel in oral and genital sites. Persistent oral HPV infection in the child was significantly associated with persistent oral HPV infection in the mother at month 36 of follow-up, hand warts in the mother, young age at onset of sexual activity for the mother, and the mother’s use of oral contraception, as well as with the father’s oral HPV status at 24 months. Persistent genital HPV infection in the infant was predicted by if the mother had started smoking at 18–21 years of age and by a history of genital warts.

Conclusions. Persistent carriage of high-risk HPV types was detected in oral and genital mucosa specimens obtained from 10% and 1.5% of the infants during their first 26 months of life. The rates of acquisition and clearance of HPV were similar in oral and genital mucosa.

Human papillomavirus (HPV) has been detected in subjects as young as newborn babies, mostly in mucosal samples obtained in the delivery room. The rates of HPV detection in nasopharyngeal aspirate specimens and oral samples vary from 0% to 67%, and in genital samples, the rates vary from 0% to 56% [1]. The presence of HPV in infants results in part from the passage of the infant through the infected birth canal. However, it is unclear whether the presence of HPV reflects passive contamination or is it a true perinatal infection of the infant [2–5]. Other possible modes of HPV transmission in infants are periconceptual transmission; prenatal transmission via placenta, amniotic fluid, and cord blood; horizontal transmission; and autoinoculation [1, 5, 6].

Persistence of HPV DNA in the oral or genital mucosa of infants may indicate true HPV infection. This was first suggested by Fredricks et al. [7] in 1993 in an article reporting that presence of HPV may persist for ≥6 weeks after delivery, indicating true infection. Subsequent studies have confirmed that more than one-half of infants still had HPV DNA present in oral and/or genital mucosa after 6 weeks of follow-up [8–10].
16 was detected in 83% of infants, but HPV type 18 was detected only in 20% of infants [10]. Earlier, we demonstrated that persistence of HPV carriage in children vary in duration from 2 days to up to 3 years [11]. However, the persistence of mucosal HPV DNA during the first years of life has not been reported in all studies [2, 12].

To our knowledge, there are no previous data on acquisition and clearance rates for mucosal HPV infection in infants. Studies of the natural history of mucosal HPV infection have mostly involved young adults [13–18]. The significance of early HPV infection detected in infants is not known, and the risks of persistent HPV carriage for infants are also unknown. The prospective Finnish HPV Family Study was designed to shed more light on the dynamics of HPV transmission within a family and to assess the modes of spread of high-risk types of HPV among infants and their parents [19]. In the present study, we present findings on the acquisition, clearance, and persistence of the oral and genital high-risk HPV DNA detected in infants during their first 26 months of life.

SUBJECTS, MATERIALS, AND METHODS

Subjects. The present study is a part of the prospective Finnish HPV Family Study [19], conducted jointly at the Department of Obstetrics and Gynecology, Turku University Central Hospital (Turku, Finland), and the Institute of Dentistry, Faculty of Medicine, University of Turku. This study includes 324 infants who have been prospectively observed since delivery for a mean duration of 25.2 months (median duration, 26.2 months). Analyses also include data for 307 mothers and 128 fathers for whom prospective follow-up data were available (mean durations of follow-up, 26.8 months and 25.7 months, respectively). The mean ages of mothers and fathers were 25.5 years (range, 18–46 years) and 28.7 years (range, 19–46 years). The parents completed questionnaires that requested demographic data and information on sexual and smoking habits, previous and current HPV infections, etc. The study protocol was approved by the Joint Commission on Ethics of University and University Central Hospital of Turku.

Samples. Oral and genital scraping samples were obtained from infants for testing for high-risk HPV DNA in the delivery room, 3 days after delivery (in the puerperal ward), and at months 1, 2, 6, 12, 24, and 36 thereafter. Oral samples were taken from both parents before delivery and at months 2, 6, 12, 24, and 36. Genital scraping samples were obtained from the mother at the same time points, and genital samples were obtained from the father only once before delivery. Scrapings were obtained for HPV DNA testing from both oral mucosa (while avoiding touching the tongue) and genital mucosa (from the labia/prepuce and scrotum) of the infant, from the cervical mucosa of the mother, and from the distal part of the urethral mucosa of the father using a small brush (Cytobrush; Med-Scand). Cervical samples were placed in phosphate-buffered saline with 100 µg of gentamycin, and all other samples were placed in 70% ethanol, as described in detail elsewhere [19].

HPV detection. DNA was extracted from scraping specimens for HPV testing as described elsewhere [19]. In short, nested PCR with MY09/MY11 and GP05+/GP06+ primers was used. Contamination was carefully monitored by simultaneous extraction of DNA from cultured human fibroblasts. Additionally, every eighth sample obtained for PCR contained no DNA. DNA dilution of SiHa cells (i.e., cells from the cervical cancer cell lines that contain 1 copy of HPV type 16) was used as a positive control for HPV DNA detection. DNA extraction, master mix for PCR, and addition of target DNA in the reaction mixture were all done in separate rooms. All PCR products were run on gel and transferred to nylon filter for hybridization with digoxigenin-labeled high-risk (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, and 58) HPV oligoprobes as a cocktail [20]. The sensitivity of the PCR method is >20 copies of HPV, because 20 SiHa cells, which contain 1 HPV type 16 copy per cell, mixed with 300 ng of human fibroblast DNA yielded a detectable signal with this method.

Definition of HPV outcomes. The dynamics of HPV infection in the oral and genital mucosa (of parents and infants) were assessed using serial samples, in accordance with the aforementioned protocol. During the follow-up period, the 5 possible outcomes for subjects in whom HPV was detected were as follows: (1) subjects remained HPV negative throughout the entire follow-up period, (2) HPV positivity developed in an originally HPV-negative subject, (3) HPV was persistently identified in all analyzed samples, (4) clearance of HPV occurred in an originally HPV-positive subject, and (5) only 1 sample was taken. The same 5 categories were used for the parents and infants. In assessing the factors that predicted the persistent presence of HPV DNA, subjects in category 3 (those with persistent carriage) were compared with subjects in category 4 (those for whom carriage cleared) to disclose the determinants distinguishing HPV persistence from clearance of the virus. This study does not include analyses of the predictors of incident HPV infections or virus clearance, which remain to be reported separately.

Statistics. Statistical analyses were performed using the SPSS computer program package, version 12.0.1. for Windows (SPSS). Frequency tables were analyzed using the χ² test and were interpreted using either the likelihood ratio test or Fisher’s exact test for determination of the significance between the categorical variables. ORs and 95% CIs were calculated as appropriate using the accurate method. Univariate survival analysis for the outcome measures (incident HPV and virus clearance) was based on Kaplan-Meier analysis with log-rank test statistics. In all analyses, the P values of <.05 were regarded to be statistically significant.
RESULTS

Detection of HPV DNA in Infants

Figure 1 shows the detection rates for high-risk HPV DNA in oral and genital samples obtained from the infants during 36 months of follow-up. At delivery, 14% of the oral and 15% of the genital samples tested positive for high-risk types of HPV. At different times during the follow-up period, the prevalence of HPV in oral and genital mucosa varied from 12% to 21% and from 4% to 15%, respectively.

Outcomes of HPV DNA in Infants

Oral mucosa. During the follow-up period (median duration 26.2 months), HPV DNA was never detected in any of the samples obtained from 37% of the infants, whereas 10% of infants had persistent oral HPV carriage, as shown in figure 2. During the follow-up period, an incident oral HPV infection was acquired by 42% of the infants, and HPV was cleared in 11%.

Genital mucosa. In the genital tract, 47% of the infants tested negative for HPV in all 8 follow-up samples, whereas incident genital HPV carriage was acquired by 36% of the infants (figure 2). At completion of the follow-up period, persistent genital HPV carriage was detected only in 5 infants (1.5%), whereas genital HPV carriage cleared during the observation period in 14% of the infants who were HPV positive at baseline.

Acquisition of Oral and Genital HPV in Infants

Kaplan-Meier analysis was used to model the acquisition of incident HPV DNA in oral and genital mucosa, as depicted in figure 3. During the 36-month follow-up period, a total of 136 and 116 infants acquired incident oral and genital HPV carriage, respectively. The survival curves demonstrating the cumulative incidence of HPV at these 2 sites run in parallel to each other, with no statistical significance ($P = .2278$, by log-rank test).

Clearance of Oral and Genital HPV in Infants

Similar analysis was used to model the clearance of oral and genital HPV during the observation period, as illustrated in figure 4. Altogether, oral and genital HPV DNA was cleared by 37 and 46 infants, respectively. As shown by the survival curves for cumulative clearance, these 2 sites did not differ from each other ($P = .3770$, by log-rank test), indicating that HPV clearance from oral and genital mucosa follows a practically identical course.

Determinants of Persistent Carriage of High-Risk HPV Types in Infants

Oral carriage of HPV. Variables recorded in the questionnaire, as well as the HPV DNA data obtained from the parents, were entered in univariate analysis to determine the predictors of persistent HPV carriage in the infant (table 1). Persistent oral HPV carriage in an infant was significantly associated with the following characteristics of the mother: oral carriage of high-risk HPV types at month 36, young age (14–16 years) at the onset of sexual activity, early age (14–16 years) at start of use of oral contraception, and hand warts. However, the mother’s age, smoking, and genital warts were not associated with persistent oral HPV carriage in the infant. Oral carriage of high-risk HPV types in the father at month 24 was the only variable to be significantly associated with persistent oral HPV carriage in the infant. No association was detected between infant’s persistent oral HPV carriage and the father’s age, smoking status, or seminal or urethral HPV status or whether he had warts.

Genital carriage of HPV. Persistent genital HPV carriage in the infant was predicted by the following: the mother had started smoking at the age of 18–21 years, and the mother had recent genital warts at the age of ≥20 years (table 1). However, neither the mother’s age, smoking history, nor hand wart status were associated with persistent genital HPV carriage in the infant. None of the father’s determinants affected persistent genital carriage of high-risk HPV types in the infant.

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Figure 3. Cumulative incidence of new human papillomavirus DNA carriage in oral samples and genital samples from 136 and 116 infants, respectively, as determined by Kaplan-Meier analysis. \( P = .2278 \), by log-rank test.

Figure 4. Cumulative clearance of oral and genital human papillomavirus DNA in 37 and 46 infants, respectively, as determined by Kaplan-Meier analysis. \( P = .3770 \), by log-rank test.

DISCUSSION

The current study shows that high-risk HPV DNA can be detected both in the oral and genital mucosa of infants during the first 3 years of life and that some HPV infections are persistent. HPV DNA has been mostly detected during the first year of infancy, reaching the peak (21%) in oral samples at 6 months of age. This increase might have resulted from both diminished maternal HPV antibodies in infants [21] and newly acquired HPV infections. In addition to vertical transmission, HPV infections might be transmitted horizontally from other family members (e.g., via caring hands or by autoinoculation) [22–24]. The present study showed a decreasing rate of carriage of high-risk HPV DNA during the first year of life, but HPV DNA was still detectable in 10% of mucosal samples obtained at the 3-year follow-up visit. This finding is in line with some earlier studies [25, 26], but the percentage of positive results is lower in the present study than in a study in which the rate of detection was 45% [27].

The results of the present study showed that 36%–42% of infants acquired high-risk HPV DNA in oral or genital mucosa, and 11%–14% of HPV DNA–positive infants cleared virus during the 3-year follow-up period. Both cumulative incidence and clearance rates ran in parallel for oral and genital mucosa. The only published study involving infants that concerned acquisition of mucosal HPV in infants did not show incident HPV carriage at all during the 6-month follow-up period [28]. However, in a study of 4–8-year-old children, 63% of children acquired oral HPV type 16 E5 or E7 DNA [29], which is even higher than the rate among <3-year-old infants in our study. There is 1 follow-up study of infants in which acquisition of HPV was determined using skin samples. Two infants were reported to have acquired HPV DNA within 1 week after delivery [30]. In the earlier published studies, mucosal HPV DNA was undetectable (at the latest) 5 weeks after delivery [12] or 6 months after delivery [8].

The present study showed that, at 3 years of follow-up, persistent HPV carriage was detected in 10% of oral and in 1.5% of genital samples.

Table 1. Maternal and paternal risk factors for persistent oral and genital carriage of high-risk human papillomavirus (HPV) types in infants.

<table>
<thead>
<tr>
<th>Infant’s HPV status, covariate</th>
<th>OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent oral HPV carriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother had oral HPV infection at month 36 of follow-up</td>
<td>5.72 (1.08–30.31)</td>
<td>.047</td>
</tr>
<tr>
<td>Mother was aged 14–16 years at onset of sexual activity</td>
<td>4.6 (1.43–15.76)</td>
<td>.022</td>
</tr>
<tr>
<td>Mother was aged 14–16 years when she started taking oral contraception</td>
<td>3.0 (1.02–8.82)</td>
<td>.016</td>
</tr>
<tr>
<td>Mother had hand warts</td>
<td>9.30 (1.39–62.22)</td>
<td>.038</td>
</tr>
<tr>
<td>Father had oral HPV infection at month 24 of follow-up</td>
<td>...</td>
<td>.022</td>
</tr>
<tr>
<td>Persistent genital HPV carriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother was aged 18–21 years when she started smoking</td>
<td>...</td>
<td>.020</td>
</tr>
<tr>
<td>Mother’s recent genital warts occurred when she was &gt;20 years of age</td>
<td>13.5 (1.57–115.93)</td>
<td>.026</td>
</tr>
</tbody>
</table>
of genital samples. In 1998, Watts et al. [2] reported a lower prevalence of HPV carriage and no persistent HPV carriage in a 3-year follow-up study. However, in one study, the rate of persistent carriage of HPV type 16 was found to be 83%, and it was 20% for HPV type 18 after 6 months of follow-up [10], and in other studies the persistence of HPV carriage varied from 27% to 56% after 6 weeks of follow-up [7–9]. The present study also indicates that the persistent carriage was more common in oral than in genital mucosa. Gingival pockets in erupting teeth may be the reservoir of oral HPV infection [31]. Overall, our results indicate that the possibility of oral HPV infection should be taken into the account when transmission and persistence of HPV DNA is studied.

An important finding in this study was that 37% and 47% of oral and genital mucosa samples, respectively, obtained from infants were HPV negative during the follow-up period. These results raise the following question: what are the mechanisms that protect these infants from HPV infection? At the moment, they are unknown, but it can be speculated that the immune defense of the infant, HLA type, the presence of HPV antibodies, and differences in host genome may protect infants from HPV infection [32].

Evaluation of the possible determinants of persistent HPV carriage in infants also suggested that oral HPV infection plays an important role in the transmission of HPV DNA between the family members. Our analysis indicated that persistent oral HPV carriage in an infant was significantly associated with persistent oral carriage of high-risk HPV types in the parents, and that HPV may be transmitted via saliva during an infant’s nursing. Similar results were found in our earlier study on the natural history of HPV carriage in spouses. Persistent oral HPV infection of the spouse increased the risk of persistent oral HPV infection in the other spouse by 10-fold [33]. It was interesting that only a mother’s warts (but not those of the father) were associated with persistent oral and genital HPV carriage. This may be because the mother plays a major role when nursing the infant during his or her first years. An interesting finding was that father’s sexual habits were not associated with persistent HPV carriage in the infant, whereas young age at the onset of the mother’s sexual activity and start of oral contraception did. There has been no prior analysis of the determinants of persistent HPV carriage in infants. However, in the study of persistent HPV carriage in young adults, the number of lifetime sex partners and the duration of oral contraceptive use were shown to be related to persistence of HPV DNA, but not the age at which the subject first had intercourse or started smoking [34]. In another study, no correlation was found between the use of oral contraceptives and the rate of persistent/recurrent HPV infection [35].

This is the first prospective, 3-year follow-up study to describe the outcome of high-risk HPV DNA carriage in infants during their first 3 years of life. The determinants of the infant’s persistent HPV DNA carriage were also analyzed. We detected a high acquisition rate but much lower clearance rate of high-risk HPV DNA in oral and genital mucosa samples obtained from infants. High-risk HPV DNA was persistently detected in 10% of infants; this was related to persistent oral HPV carriage in the parents and to hand warts on the mother. These results help one understand the natural history of HPV carriage in young children. This information is necessary to help HPV vaccine studies focus on the correct age groups and populations for vaccination, as well as with regard to legal issues, to decrease the number of lawsuits related to false allegations of sexual abuse.

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