Human Papillomavirus Genotypes Present in the Oral Mucosa of Newborns and their Concordance with Maternal Cervical Human Papillomavirus Genotypes

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Objectives To elucidate the concordance of human papillomavirus (HPV) genotypes between the mother and her newborn and to identify risk factors for the vertical transmission of HPV.

Study design HPV genotypes present in 329 pregnant women, their newborns, cord blood, and placenta samples were determined by molecular techniques, including using pure DNA for nested polymerase chain reaction. HPV antibodies were tested using multiplex HPV serology. Kappa statistics and the Wilcoxon test were used to assess concordance, and regression analysis was used to calculate ORs and 95% CIs.

Results HPV DNA was detected in 17.9% of oral samples from newborns and in 16.4% of the cervical samples of the mothers. At delivery, mother-newborn pairs had similar HPV-genotype profiles, but this concordance disappeared in 2 months. Oral HPV carriage in newborns was most significantly associated with the detection of HPV in the placenta (OR = 14.0; 95% CI, 3.7-52.2; $P = .0001$). The association between status of the cord blood and oral HPV was also significant at delivery (OR = 4.7; 95% CI, 1.4-15.9; $P = .015$) but disappeared within 1 month. HPV antibodies in infants were of maternal origin (OR = 68; 95% CI, 20.1-230.9; $P = .0001$).

Conclusions HPV is prevalent in oral samples from newborns. The genotype profile of newborns was more restricted than that of the maternal cervical samples. The close maternal-newborn concordance could indicate that an infected mother transmits HPV to her newborn via the placenta or cord blood. *(J Pediatr 2012;160:837-43)*.

Human papillomavirus (HPV) DNA has been found in the oral mucosa of newborns and older children.[1-3] Although HPV infection traditionally is considered a sexually transmitted infection, there is a growing body of evidence supporting the existence of nonsexual transmission routes as well. Vertical transmission from an infected mother to her infant during childbirth is a candidate pathway for infants to acquire HPV.[4-8] HPV transmission during pregnancy or during the perinatal period could be one alternative transmission route, with transmission taking place through the infected placenta or cord blood or through ascension from or passage through the infected birth canal.[8-11] Contradictory opinions exist on the frequency of vertical transmission, with reported wide variations from 1% to 80%.[4,6,7,12,13] Persistent oral HPV infections in infants have been reported but, again, the variation is wide, from 1% to 83%, for follow-up times ranging from 6 weeks to 24 months.[6,14,15] Possible maternal predictors for HPV infection in infants include the HPV status of the mother, past use of hormonal contraception, a history of immunosuppression, and genital HPV diseases.[7,12-14] The HPV genotype distribution in the oral mucosa of newborns[7,12-14,16] has been studied but only using a limited panel of HPV genotypes. The present study is part of the Finnish Family HPV Study designed to assess the dynamics of HPV infections in families. Herein, we describe the HPV genotypes present in the oral mucosa of newborns during the 2-month perinatal period and the concordance of these genotypes with those present on the mother's cervix before delivery. The HPV status of the infants was related to the HPV status of the placenta and of the umbilical venous cord blood as well as with the maternal demographic data and HPV serostatus before delivery.

Methods

The Finnish HPV Family Study is a longitudinal cohort study conducted at the Department of Obstetrics and Gynecology, Turku University Central Hospital, and the Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland. The study plan was approved by the Research Ethics Committee of Turku University Hospital (#3/1998). A total of 329 pregnant women in their third trimester of pregnancy and, later, their

HPV	Human papillomavirus
HR-HPV	High-risk human papillomavirus
LR-HPV	Low-risk human papillomavirus
MFI	Median fluorescence intensity
PCR	Polymerase chain reaction

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newborns (n = 331; includes 2 sets of twins) were enrolled in the study between 1998 and 2001, as described previously.[6,17,18] Written informed consent was obtained from all participants. All of the mothers in this study were of Caucasian origin, had the same ethnic background, and were representative of the Finnish population. The median age of the mothers was 25.5 years (range, 18-46 years). Most of the mothers (73.9%, 243 of 329) had vaginal deliveries.

During their first visit after delivery, all women completed a questionnaire on their sexual behavior, gynecologic, and obstetric history as well as other risk factors of HPV infections, as previously described.[19]

A routine Pap smear was taken from all women at baseline (before delivery) using the conventional 3-sample technique with 2 wooden spatulas and a brush (Cytobrush, Medscand Medical, Malmö, Sweden). Scrapings for HPV DNA testing were taken from the cervical mucosa of the mother before delivery and the oral mucosa of the newborn (at delivery, day 3, and months 1 and 2) using a cytobrush (Medscand Medical) as described earlier.[19] Blood samples were collected from the mothers at baseline before delivery and from the newborns at the ages of 1 and 2 months. The samples were centrifuged (2400 rpm, 10 minutes), and the serum samples were divided into three 1-mL tubes, frozen at −20°C, and then stored at −70°C until serologic analysis. Umbilical venous cord blood samples were collected into vacuum ethylenediaminetetraacetic acid tubes after delivery, before the placenta was removed. Tubes were frozen at −70°C. Two placental samples from the central part of the maternal side of the placenta were taken in the delivery room immediately after delivery[20] and stored at −70°C until HPV testing. Breast milk samples were collected as described previously by Sarkola and colleagues.[21] Briefly, samples were collected manually by mothers 3 days after delivery into 3-mL plastic containers after washing their hands with disinfectant. Containers were frozen and stored at −70°C.

DNA from the umbilical cord blood and milk samples was extracted using the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions, as described earlier.[20,21] The high salt method was used to extract DNA from cervical and oral scrapings and from placental samples with a size of approximately 1 cm.[2,22] Originally, HPV testing was first done to detect high-risk HPVs without identifying the specific HPV genotypes using nested polymerase chain reaction (PCR) with the MY09/MY11 and GP05$^+$/GP06$^+$ primers for all other samples except cervical samples, for which single PCR with the GP05$^+$/GP06$^+$ primers was used, as described earlier.[21,23] Evaluation of possible contamination during the DNA extraction was performed by simultaneous extraction of DNA from cattle lung tissue and/or cultured fibroblast. The HPV-negative immortalized human gingival keratinocytes cell line was used as a negative control for the umbilical cord blood samples. Also, the HPV-positive CaSki (Human cervical epithelial carcinoma, CRL-1550, ATCC collection; LGC Promochem, Middlesex, United Kingdom) and SiHa (Human cervical epithelial carcinoma, HTB-35, ATCC collection) cell lines and no-DNA samples were used as positive and negative controls, respectively, in the nested PCR. PCR products were electrophoresed on 2.0% agarose gels (DNA agar, MBI, Derwentway Delta, British Columbia, Canada) and hybridized with a digoxigenin-labeled, high-risk HPV-oligoprobe cocktail (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 54, 56, and 58).

After HPV DNA detection using nested PCR, HPV genotyping of all the cervical and oral scrapings was performed using the Multimetrix assay (Multimetrix, Regensburg, Germany) as described earlier.[19,24] This assay detects the low-risk (LR)-HPV types 6, 11, 42, 43, 44, and 70 and the high-risk (HR)-HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. The assay was performed according to manufacturer's instructions except that only half of the volume was used. In the final step, 100 μL of the blocking buffer was used for reading the absorbance with a Luminex LX-100-analyzer (Bio-plex 200 System; Bio-Rad Laboratories, Hercules, California).[19] As target DNA, the PCR products from HPV testing, which were now biotinylated by reamplification with GP05$^+$/biotin-GP06$^+$ primers, were used. The median fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cut-off value for each HPV probe individually was defined as 1.5 × MFI (negative control) + 5 MFI.

Blood samples were sent for analysis to DKFZ (Heidelberg, Germany).[19] Serum antibodies for the major capsid protein L1 of HPV types 6, 11, 16, 18, and 45 were analyzed using multiplex HPV serology based on glutathione *S*-transferase fusion-protein capture on fluorescent beads.[25,26] Sera were scored as positive when the antigen-specific MFI values were greater than the cut-off level of 200 or 400 MFI for L1 antigens of individual HPV types.[27] The HPV seroepidemiology of the mothers during the follow-up period has been published previously.[19]

Statistical Analyses

The SPSS (SPSS Inc, Chicago, Illinois) and STATA (Stata Corp, College Station, Texas) software packages (PASW Statistics for Windows, version 18.0.1, and STAT/SE 11.1) were used for statistical analyses. Frequency tables were analyzed using the χ^2 test or Fisher exact test (when appropriate) with Pearson *R* or likelihood ratio statistics to assess the significance of the correlation between categorical variables ORs with their 95% CIs were calculated where appropriate. Differences in the means of continuous variables between the strata were analyzed using ANOVA or nonparametric tests (Mann-Whitney, Kruskal-Wallis). For all analyses, values of $P < .05$ were regarded as statistically significant.

Results

HPV was detected in 22.5%, 13.0%, 18.7%, and 16.9% of the oral samples from the whole cohort of newborns at delivery, day 3, month 1, and month 2, respectively. At the third