The possibility of vertical transmission of human papillomavirus through maternal milk

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Summary

Objective: Human papillomavirus (HPV) DNA has been detected in the oral cavity of infants and breast cancer tissue, suggesting its vertical transmission through maternal milk. We determined whether HPV is detected in maternal milk and is vertically transmitted by breast feefing.

Methods: Informed consent was obtained, and maternal milk samples (n = 80) were analyzed for high-risk HPV DNA. In 43 women, this DNA was measured in the uterine cervix. In women with positive samples, this DNA was measured in the oral cavities of their children. The domain including HPV E6 and E7 was amplified by polymerase chain reaction using consensus primers, and HPV serotype determined by electrophoresis after restriction enzyme digestion.

Results: High-risk HPV-16 was detected in 2 of 80 samples (2.5%), and in these 2 cases, high-risk HPV was not detected in the uterine cervix or oral cavity of the child.

Conclusion: The infection of HPV in maternal milk is rare (2/80), vertical transmission

through maternal milk was not detected in this study (0/80). HPV infection through maternal milk may occur, but its likelihood is low.

Introduction

The role of HPV in the pathogenesis of human cancers has been studied, and two HPV genes are known to encode the oncoproteins E6 and E7. The E6 and E7 proteins of HPV16 and 18 have been demonstrated to be necessary to efficiently immortalize their natural host cells (Kaur et al., 1989). HPV types 16 and 18 are known to be carcinogenic, and HPV types 31 and 33 are also likely carcinogenic in human cervical and anogenital cancers (International Agency for Research on Cancer, 1995). Approximately 40 distinct HPV types are known to infect the genital tract, and epidemiological studies to date suggest that at least 14 of these, called oncogenic or high-risk types, are significantly associated with progression to invasive cervical cancer (Bosch et al., 1995). Most of these high-risk types are phylogenetically related to either HPV16 (31, 33, 35, 52 and 58) or HPV18 (39, 45, 59 and 68) (Chan et al., 1995). Evidence indicates that HPV can be present in breast tissue, and therefore may also be present in maternal milk. HPV replication in primary human mammary ductal epithelial

cells has been demonstrated, and HPV types 16 and 18 can immortalize normal breast epithelium *in vitro* (Wazer et al., 1995). Contradictory results concerning HPV detection in breast carcinomas have been reported (Syrjänen & Syrjänen, 2000).

Several viruses have been detected in human maternal milk, and some viruses such as HTLV-1 (human T-lymphotropic virus type 1) and HIV (human immunodeficiency virus) can infect newborns (Michie & Gilmour, 2001). HPV is known to infect the oral mucosa of infants (Wazer et al., 1995), and may also infect the epithelium of the nipple and areola (Syrjänen & Syrjänen, 2000). The Finnish HPV Family Study is a prospective follow-up cohort study started 1998 to evaluate the transmission modes of HPV between newborns and their parents (Rintala et al., 2005). In this study, HPV DNA was detected in 15% of the genital and 10% of the oral samples collected from infants at birth, reaching peaks of 18% and 21%, respectively, at 6 months, and declining to 10% at 24 months (Rintala et al., 2005). However the infection of HPV in maternal milk has not been studied so far. Here we present the results of HPV

DNA testing in breast milk samples collected 3–7 d after delivery and incidence of HPV infection of infant oral cavity, to establish vertical HPV transmission through milk.

Materials and methods

Subjects

80 women who delivered at Tokushima University Hospital participated in this study. Informed consent was sought and obtained from all participants. With the approval of the Ethical Review Board of Tokushima University Hospital, 80 samples of maternal milk and 43 samples of uterine cervix were analyzed for high-risk HPV DNA. The mean age of mothers was 30.6 years (range, 19–42 years). In addition, the children of women with positive breast milk samples were examined for high-risk HPV DNA in the oral cavity at 15 months after birth, when their mothers finished nursing, to exclude the possibility of contamination by nursing. The children of women with positive cervical samples were also examined for high-risk HPV DNA in the oral cavity at 3 months after

birth.

Samples and DNA extraction

At the hospital 3–7 d postpartum, each mother collected a 5-ml milk sample manually into a 15-ml plastic centrifuge tube after washing hands with disinfectant. This procedure was done separately from feeding the infant. Cervical scrapings were collected 1 month after delivery. Cervical samples were collected with a brush, and oral cavity samples with cotton swabs. The samples were immediately frozen and stored at -80ºC. For DNA extraction, samples were suspended in digestion buffer containing proteinase K, and subsequently handled and processed in the same manner described below.

The milk samples were centrifuged for 20 min at 3500 rpm to pellet the cells, from which DNA was extracted. Great care was taken during handling to avoid any contamination between samples. The pellet was suspended in digestion buffer (100 mM Tris-HCL pH 8.0, 0.5 M EDTA, 10% SDS) containing 20 mg/ml of proteinase K

(Sigma-Aldrich, USA) and incubated for 12 h at 37ºC. DNA was extracted in phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v) and resuspended in 20 µl distilled water. DNA concentration was determined with a spectrophotometer at 260 nm.

Polymerase chain reaction (PCR)

PCR amplification was applied to the analysis of breast milk, cervical, and oral samples using the same method, as previously described (Watts et al.,1998). For PCR amplification, 0.5 µg extracted total DNA was used as a template. All PCR procedures were performed in a final volume of 50 µl under identical conditions. The first PCR amplification was performed with HPV E6 and E7 consensus primer pair (HPVpU-1M forward primer 5′-TGTCAAAAACCGTTGTGTCC-3′ and HPVpU-2R reverse primer 5′-GAGCTGTCGCTTAATTGCTC-3′) (22.5 pmol each), using 1.25 U AmpliTaq DNA polymerase (TaKaRa Ex Taq^{TM}), PCR buffer (10×Ex Taq Buffer, Mg^{2+} concentration 20 mM), dNTP mixture (200 μM). The PCR mixture was subjected to 30 cycles of amplification consisting of denaturation at 94ºC for 1 min, annealing at 54ºC for 45 s,

and elongation at 72ºC for 1 min. The domain including HPV E6 and E7 was amplified by PCR with consensus primers (TaKaRa PCR Human Papillomavirus Typing Set), with which high-risk HPV-16, 18, 31, 33, 35, 52, and 58 can be amplified (Fujinaga et al., 1991). HPV type was determined by electrophoresis after restriction enzyme digestion (AvaII, AfaII, BglII, AccII, AvaI; Enzyme Set A, TaKaRa). To control for the quality of extracted DNA, the β-actin gene was first amplified with PCR in 32 milk and oral cavity samples using previously described primers (Dai et al., 2005), and the same amount of β-actin product was obtained for each sample by running the same volume of product on an agarose gel.

Results

Characteristics of the enrolled women are summarized in Table 1. The median age at delivery was 30.6 years (range, 19–42 years). The mean number of gravidities was 2.3 (range, 1–7), and parity was 1.8 (range, 1–6). We initially tested the presence of

high-risk HPV DNA in breast milk. High-risk HPV DNA was detected in 2 of 80 milk samples (2.5%) in the cellular compartment (Figure 1). Sequencing indicated that both HPV-positive samples contained HPV-16-DNA. The women with HPV DNA-positive maternal milk were both negative for high-risk HPV DNA in cervix, and they had no history of HPV-related disease (condylomata, cervical intraepithelial neoplasia, or cervical cancer) or mammary disease (Table 1).

Table 2 summarizes the HPV types and prevalence in mothers and infants (specimens collected 3 or 15 months after birth). In cases of HPV DNA-positive maternal milk, HPV DNA was not detected throughout the buccal mucosa of children 15 months after birth. In cases of HPV DNA-positive cervix, HPV DNA was not detected in the oral cavity of children 3 months after birth (Figure 1). All 4 patients with cervical HPV delivered by vaginal. β-actin was detected by PCR in all samples from children, indicating that these samples contained sufficient DNA for analysis.

Discussion

HPV types 16 and 18 are carcinogenic, and HPV types 31 and 33 are likely also carcinogenic, in human cervical and anogenital cancers (International Agency for Research on Cancer, 1995). Connections between HPV and breast cancer have also been reported (Di Lonard et al., 1992, Yu et al.,1999, Hennig et al., 1999, Liu et al., 2001). Damin et al. detected HPV in 25 of 101 (24.75%) patients with breast cancer, and did not identify HPV in any of 41 benign lesions (20 patients with reduction mammoplasty and 21 with mammary fibroadenomas). The authors isolated HPV-16 DNA in 14 patients (56%), HPV-18 in 10 patients (40%), and both serotypes in one patient (4%) (Damin et al., 2004).

Vertical transmission of HPV has also been reported. In 1993, Fredericks and colleagues reported the transmission of HPV from mother to child in 8 of 11 women with genital genotypes of HPV in their cervical smears. The children had HPV of the same genotype in buccal mucosal cell samples, and one child whose mother had no

HPV DNA detected in her cervical smears was also positive for HPV DNA (Fredericks et al., 1993). In 1994, Pakarian et al. found HPV DNA in buccal or genital swabs from 3 of 11 infants born to women with HPV DNA-negative cervical swabs. In another study by Puranen et al., HPV DNA was found in 31 of 98 (31.6%) oral scrapings from children, 12 of whom were born to mothers negative for HPV DNA in uterine cervix (1996). Watts et al. reported positive specimens in 3 of 80 infants (4%) born to women with HPV DNA detected at 34 weeks' gestation and 5 of 63 infants (8%) born to women without HPV DNA (1998). In these reports, HPV type was not always concordant between newborn and mother (Smith et al., 2004). In infants, HPV DNA was detected in 15% of genital and 10% of oral samples collected at birth, reaching peaks of 18% and 21%, respectively, at 6 months, and declining to 10% at 24 months (Rintala et al., 2005). These findings suggest the possibility of vertical transmission through maternal milk, in addition to other infection pathways via the birth canal or the oral cavity of parents.

The results of the present study support the possibility that HPV infection can

occur through maternal milk. The origin of HPV DNA in the mother's milk remains an interesting question. The cellular compartment of human milk contains epithelial cells as well as cells of the immune system. According to current opinion, HPV infects epithelial cells and multiplies locally at the site of entry on the skin or mucous membranes, and infection does not lead to viraemia. A suggestive clue comes from the observation by de Villiers et al. (2006) that HPV is present in cancers occurring in human nipple milk ducts, and that these cancers have the typical histological features of HPV-induced human cancers. This finding supports the view of a pathogenic mechanism involving HPV transfer in a retrograde fashion via nipple, areola, lactiferous ducts, and sinuses.

Even when HPV infects the uterine cervix, it is thought to disappear naturally in about 90% of cases. Persistent infection is related to CIN or cervical cancer. In a similar manner, if HPV infection of a breast should occur through a nipple in a sexual encounter, HPV may be removed naturally in many cases, and persistent infection may be related

to the malignant transformation of breast cancer. Although our results support the possibility of HPV infection through maternal milk, this phenomenon suggests that even if HPV is contagious temporarily to the oral mucosa of the child, it will also be removed naturally in many cases. This study indicates that the risk of HPV infection in maternal milk is low and vertical transmission of HPV via maternal milk is probably low.

Declaration of interest

The authors do not have any conflicts of interest to disclose.

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Figure legend

Figure 1

Mother: PCR was performed using the primer pair HPVpu-1M/HPVpu2R. Lane 1: high-risk HPV positive control; lane 2: negative control; lane 3: milk sample (case 1: positive); and lane 4: milk sample (case 2: positive). *Child*: Lane 1: high-risk HPV positive control; lane 2: oral sample (case 1: negative); lane 4: oral sample (case 2: negative); lane 6: oral sample (case 3: negative); lane 8: oral sample (case 5: negative); lane 10: oral sample (case 6: negative); and lanes 3, 5, 7, 9, and 11: β-actin was amplified (cases 1, 2, 3, 5, and 6, respectively).

Table1. Maternal characteristics at enrollment and HPV infection in maternal milk (N=80)

Maternal Characteristics	No. n=80	HR HPV-positive mothers in beast milk (%) n=2
Age		
<25	7	0 (0)
25-34	55	2 (3.6)
35≤	18	0 (0)
Gravidity		
1	25	2 (8.0)
2 ≤	55	0 (0)
Parity		
1	34	2 (5.9)
2 ≤	46	0 (0)
Smoking		
Never	73	2 (2.7)
Ever	7	0 (0)
HPV infection in cervix		
Negative	39	2 (5.2)
Positive	4	0 (0)
No sample	37	0 (0)
HPV-related disease history		
Never	79	2 (2.5)
CIN	1	0 (0)
Type of delivery		
Vaginal	60	1 (1.7)
Cesarean-section	20	1 (5.0)
Mammary disease history		
Never	78	2 (2.6)
Mastitis	2	0 (0)

Table 2. HPV prevalence and types in mothers and infants

Case	Mother		Baby	Complication
	Breast milk	Cervix	Oral	
1	HPV16	N	N	EBV infection
2	HPV16	N	N	
3	N	HPV52	N	
4	N	HPV16	—	
5	N	High-risk HPV	N	
6	N	High-risk HPV	N	

"—" = no sample available N = negative
Oral = oral sample in 3 or15 months after birth
Breast milk = breast milk sample in 3-7days after delivery
Cervix = cervix sample in 1 month after delivery