ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803–5253

REGULAR ARTICLE

Can 'high-risk' human papillomaviruses (HPVs) be detected in human breast milk?

Ioannis N Mammas (mammasjo@googlemail.com)[1], Apostolos Zaravinos[2], George Sourvinos[2], Nikolaos Myriokefalitakis[1], Maria Theodoridou[3], Demetrios A Spandidos[2]

1.1st Department of Paediatrics, 'Penteli' Children's Hospital, Athens, Greece
2.Department of Clinical Virology, School of Medicine, University of Crete, Heraklion, Greece
3.1st Department of Paediatrics, 'Aghia Sophia' Children's Hospital, University of Athens School of Medicine, Athens, Greece

Keywords
Breast, Children, Human papillomavirus, Milk, Polymerase chain reaction

Correspondence
Dr IN Mammas, M.D., Ph.D., 1st Department of Paediatrics, 'Penteli' Children's Hospital, Athens, Greece.
Tel./Fax: +30 213 2052362 |
Email: mammasjo@googlemail.com

Received
21 September 2010; revised 22 November 2010; accepted 15 December 2010.

DOI:10.1111/j.1651-2227.2010.02129.x

ABSTRACT

Aim: Human papillomavirus (HPV) transmission via nonsexual modes in childhood has been proposed by several researchers. The aim of our study was to determine the presence of 'high-risk' HPV DNA in human breast milk.

Methods: Using polymerase chain reaction techniques, we evaluated the presence of HPV infection in human breast milk collected from 21 HPV-positive and 11 HPV-negative mothers.

Results: Of the 32 studied human milk specimens, no 'high-risk' HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 or 58 DNA was detected.

Conclusion: This preliminary case–control study indicates the absence of mucosal 'high-risk' HPV types in human breast milk.

INTRODUCTION

Human papilloma viruses (HPVs) are small double-stranded DNA viruses associated with a wide range of cutaneous and mucosal infections in adults as well as in children (1,2). Mucosal HPV types 16 and 18 represent the most frequent 'high-risk' types that are detected in the female anogenital system and are present in more than 70% of women with cervical cancer (3). The recently introduced vaccination programme against HPV in childhood is expected to prevent HPV-16- and HPV-18-related cervical cancer in women. Although HPV infection is considered a sexually transmitted infection, HPVs can also be transmitted by nonsexual routes including casual physical contact and perinatal vertical transmission (4–7).

Vertical mother-to-infant transmission of HPV has been proposed by various studies, including the mother-to-infant transmission via breast milk (5,7). Recently detection of HPV infection in the buccal cavity of healthy children has raised new questions concerning the vertical mode of HPV transmission in childhood via breast milk (8). The presence of HPV in breast milk can potentially increase the apprehension of mothers with HPV-related cervical neoplasia preventing the breast feeding of their infants. Since to date data on the presence of HPV in human breast milk among women with genital HPV infection are limited, we conducted a case–control study to determine the presence of 'high-risk' HPV DNA in human breast milk (9).

SUBJECTS AND METHODS

Human breast milk specimens were collected from 11 HPV-negative mothers with normal Papanicolaou smear test and 21 mothers with HPV-associated lesions, who attended the 'Penteli' Children's Hospital, in Athens, Greece, during the period January 2009–2010. Consent form was acquired by all women included in our study.

The milk samples were centrifuged for 20 min at 146.9 g to pellet the cells, from which the DNA was extracted with the ZR Genomic DNA kit according to the manufacturer's specifications (Zymo Research, Orange, CA, USA). The DNA concentration was calculated using the NanoDrop™ 1000 Spectrophotometer. Specimens were examined for the presence of amplifiable DNA using a set of primers for the *β2-microglobulin* gene. Twenty nanograms of genomic DNA was applied to each polymerase chain reaction (PCR), and all samples were run in duplicate. The samples were initially examined for the presence of nontype-specific HPV DNA using the general primers GP5+/6+ and the 2× Brilliant SYBR-Green I QPCR master mix (Stratagene, La Jolla, CA, USA) (10). After initial denaturation at 95°C for 7 min, samples were subjected to 45 amplification cycles comprised of denaturation at 95°C for 10 sec, annealing at 45°C for 35 sec and elongation at 72°C for 30 sec, followed by a melt curve analysis in which the temperature was increased from 55 to 95°C at a linear rate of 0.2°C/sec. Appropriate negative and positive controls were included in each PCR to

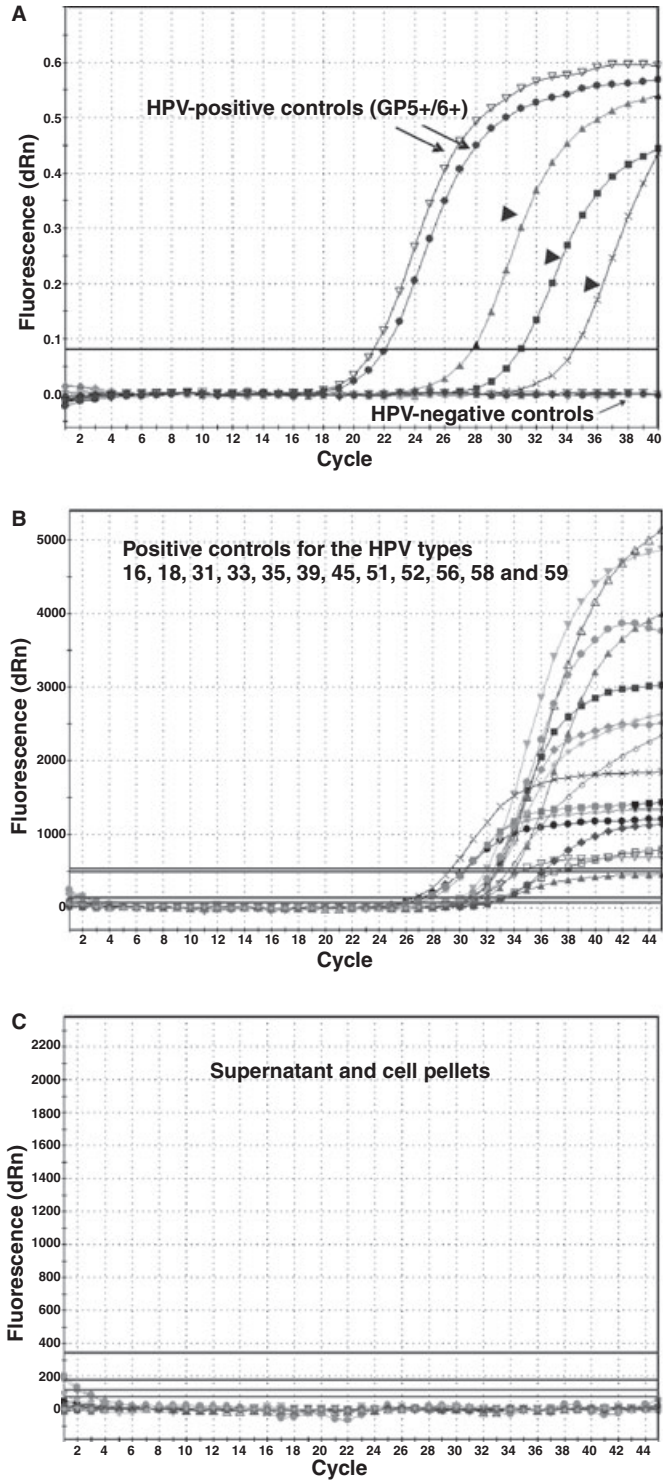

Figure 1 Detection of HPV DNA in representative milk samples included in our study. The breast milk samples were divided into supernatant and cell pellets, and were initially examined for the presence of HPV DNA using the general GP5+/6+ primers. (A) Amplification plot depicting HPV typing for the GP5+/6+-positive samples. Arrows in the amplification plot show the positive and negative control samples, respectively. Arrowheads depict three serial dilutions (2-fold) of an HPV-positive cell pellet sample. HPV-negative controls did not show amplification curve. (B) Amplification plot of the positive controls for the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. (C) Amplification plot depicting that none of the supernatant or cell pellet samples was positive for the studied 'high-risk' HPV types.

exclude contamination events and to establish the specificity of primer-directed amplification.

For the general screening of HPV DNA, HeLa cells transfected with conserved L1 sequences among the HPV strains were used as the positive control. Downstream HPV-typing was performed for the HPV-positive sample with the HPV HighRisk Typing Real-TM kit (Sacace Biotechnologies, Commo, Italy), according to the manufacturer's recommendations This kit can detect qualitatively and genotype the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. *β-globin* was used as internal control. Statistical analysis was performed using the SPSS computer program package (version 12.0.1 for Windows).

RESULTS

The mean ages of the HPV-negative mothers with normal Papanicolaou smear tests and the mothers with HPV-associated lesions were 25 (range 19–28 years) and 26 years (range 20–32 years), respectively ($p = 0.345$, nonstatistically significant). Among the women with HPV-associated lesions, five women had 'high-grade' squamous intraepithelial lesions (SILs) and 16 had 'low-grade' SILs. All neonates delivered by the mothers included in our study did not exhibit any HPV-associated skin or mucosal lesions. Analysis of breast milk demonstrated the presence of cellular DNA in all specimens. Only one cell pellet sample was HPV positive using the general primers GP5+/6+. This breast milk sample was collected from an HPV-negative mother with normal Papanicolaou smear test. Further analysis with the HPV HighRisk Typing method did not show positivity for any of the 12 studied 'high-risk' HPV types. None of the supernatant samples was HPV positive (see Fig. 1).

DISCUSSION

Our study provides further evidence of the absence of 'high-risk' HPV DNA in human breast milk collected from women with HPV-associated lesions. According to the literature, the presence of HPV in human breast milk was found in only one study performed in 2008 by Sarkola et al. (9). In this study, HPV-16 DNA was detected in 4% of the studied breast milk samples implicating the possible role of breast feeding in the mother-to-infant transmission of HPV. However, in our study, 'high-risk' HPVs were not detected in any of the studied cases supporting vertical transmission via breast milk as an unlikely mode of 'high-risk' HPV transmission.

Notably, our findings were relevant to the results of the study by Sarkola et al.(9) who demonstrated no relationship between maternal cervical and breast milk HPV infection. A further search of the literature did not provide any primary evidence to support the involvement of breast feeding in HPV transmission (5). The presence of HPV in breast milk and its possible clinical relevance regarding HPV transmission in children require further clarification in larger scale case–control studies. At present, no evidence exists to support the avoidance of breast feeding owing to the possible mother-to-infant transmission of HPV via breast milk.

References

1. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; 2: 342–50.
2. Mammas I, Sourvinos G, Spandidos DA. Human papilloma virus (HPV) infection in children and adolescents. *Eur J Pediatr* 2009; 168: 267–73.
3. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2002; 88: 63–73.
4. Armstrong DK, Handley JM. Anogenital warts in prepubertal children: pathogenesis, HPV typing and management. *Int J STD AIDS* 1997; 8: 78–81.
5. Syrjanen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. *Crit Rev Oral Biol Med* 2002; 11: 259–74.
6. Myhre AK, Dalen A, Berntzen K, Bratlid D. Anogenital human papillomavirus in non-abused preschool children. *Acta Paediatr* 2003; 92: 1445–152.
7. Cason J, Mant C. High-risk mucosal human papillomavirus infections during infancy & childhood (Review). *J Clin Virol* 2005; 32S: S52–8.
8. Mammas I, Sourvinos G, Michael C, Spandidos DA. Human papilloma virus in hyperplastic tonsillar and adenoid tissues in children. *Pediatr Infect Dis J* 2006; 25: 1158–62.
9. Sarkola M, Rintala M, Grenman S, Syrjanen S. Human papillomavirus DNA detected in breast milk. *Pediatr Infect Dis J* 2008; 27: 557–8.
10. de RodaHusman AM, Walboomers JM, van der Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995; 76: 1057–62.