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Distribution of maternal and infant human papillomavirus: risk factors associated with vertical transmission

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ABSTRACT

Objective: To evaluate the rate of human papillomavirus (HPV) infection in pregnant women and their neonates, and the risk factors associated with vertical transmission of HPV infection from mothers to neonates.
Study design: Cervical HPV testing was undertaken in pregnant women over 36 weeks of gestation, and mouth secretions and oral mucosa of neonates were tested for HPV immediately after delivery. HPV-positive neonates were rechecked 2 months postpartum to identify the persistence of HPV infection. In HPV-positive mothers, the placenta, cord blood and maternal peripheral blood were also analysed for HPV to confirm whether transplacental HPV infection occurred.
Results: HPV was detected in 72 of 469 pregnant women (15.4%) and in 15 neonates (3.2%). Maternal HPV positivity was associated with primiparity and abnormal cervical cytology. The rate of vertical transmission was 20.8%, and all HPV-positive neonates were born from HPV-positive mothers. Vertical transmission was associated with vaginal delivery and multiple HPV types in the mother. Neonates with HPV showed a tendency for higher maternal total HPV copy number than neonates without HPV, but this difference was not significant ($p = 0.081$). No cases of HPV infection were found in the infants at 2 months postpartum, and no HPV was detected in placenta, cord blood or maternal blood.
Conclusions: Vertical transmission of HPV is associated with vaginal delivery and multiple HPV types in the mother; however, neonatal HPV infection through vertical transmission is thought to be a transient.

1. Introduction

Human papillomavirus (HPV) is a sexually transmitted virus that gives rise to anogenital warts and cervical cancer in adults [1], and laryngeal papilloma, conjunctival papilloma and recurrent respiratory papillomatosis in children [2–6]. Recurrent respiratory papillomatosis is transformed into malignant laryngeal carcinoma in 3–5% of cases [6]. HPV infection is usually caused by sexual intercourse, but non-sexual transmission has also been reported, including vertical transmission from mother to neonate, horizontal transmission from those in close contact with the neonate including other family members, auto-inoculation from one infection site to another, and indirect transmission through contaminated objects [7].

The rate of HPV infection in pregnant women ranges widely from 6% to 65%, with an average of approximately 24%. The rate of vertical transmission of HPV from mother to neonate also ranges widely from 4% to 72% depending on regional and study population differences [8,9].

This prospective study reports the rate of vertical transmission of HPV infection in pregnant women in Korea. The study aimed to evaluate the rate of HPV infection in pregnant women, and identify the risk factors associated with vertical transmission. In addition, the study evaluated whether neonatal HPV infection is acquired through transplacental transmission during pregnancy.

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2. Materials and methods

2.1. Study population

Pregnant women over 36 weeks of gestation who planned to give birth at the Cheil General Hospital and Women's Healthcare Centre were recruited between March 2010 and April 2011. The institution's ethical committee approved this research, and all the participants gave informed consent to participate. Women with severe intrauterine growth restriction, severe oligohydramnios, preterm premature rupture of membranes, severe hypertension during pregnancy, a history of malignancy within the last 5 years, liver cirrhosis, renal failure, chronic disease affecting maternofetal immunity, a history of psychological disease and alcoholism were excluded. The rate of vertical transmission, and demographic and reproductive characteristics associated with vertical transmission were analysed, and the HPV viral loads of HPV-positive mothers were measured to investigate the relationship between vertical transmission and viral load.

2.2. Samples

Cervical HPV testing was undertaken in the pregnant women, and mouth secretions and oral mucosa of the neonates collected immediately after delivery were tested for HPV. HPV-positive neonates were rechecked at 2 months postpartum to verify persistence of the HPV infection. In HPV-positive mothers, the placenta, cord blood and maternal peripheral blood were also analysed for HPV to confirm whether transplacental HPV infection occurred.

Maternal peripheral blood was taken before delivery, and placenta and umbilical venous cord blood were obtained immediately after delivery. A 2 × 2 cm sample of central placenta including all tissue layers was taken, and the samples were fixed in neutral 10% formalin and processed in paraffin blocks.

2.3. Isolation of DNA

DNA from exfoliated cervical cells, placenta and neonate oral mucosa was isolated using a QIAGEN tissue genomic DNA Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. DNA from cord and maternal peripheral blood was isolated using a QIAamp Blood Mini Kit (QIAGEN) according to the manufacturer's instructions.

2.4. SYBR green I real-time polymerase chain reaction (PCR)

SYBR Green I real-time PCR reactions were performed with 4 pmol GPM 7 forward primers (F1, F2), 8 pmol Cy5-labelled reverse primer (*R*) and 2 μl DNA sample, containing 10 μl of 2X SYBR Green I Master Mix (Roche Diagnostics GmbH, Mannheim, Germany) and 0.5 units uracil DNA glycosylase in a 20 μl reaction. The nucleotide sequences (5′–3′) of GPM7 F1 and F2 are AGTGGT CATCCWTTWTT-WAATAAATTKGATGA and AGTGGCCATCCWTTDTWKAA TAG-GYWKGATGA, respectively. The nucleotide sequence of GPM R (5′–3′) is Cy5-CCAWAGCCWGTATCWACCATRTCACCATC. SYBR Green I real-time PCR conditions for human beta globin were the same except for the primer condition, which was conducted with 5 pmol GPM F1, F2 and Cy5-labelled GPM R.

2.5. Cheil HPV DNA chip assay

Each product (5 μl) from the SYBR Green I real-time PCR of HPV and human beta globin was denatured in a single tube at 95 °C for 10 min, and transferred immediately to ice. The mixture containing real-time PCR products and hybridization buffer (5X SSC, 0.2% SDS) was injected slowly into the hole of cover slips on the prepared slides. Hybridization was performed at 60 °C for 30 min under high humidity. After hybridization, the slides were washed three times with washing solution (1X SSC, 0.1% SDS) and rinsed three times with 1× SSC. After drying the slides, HPV types were identified using a chip scanner (NimbleGen MS 200, Roche NimbleGen, Switzerland) and analysed with GenePix Pro Version 6.0 (Axon Instruments, Inc., Union City, CA, USA). The Cheil HPV DNA chip can detect the presence of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 68a and 82 (high-risk types) and HPV 6, 11, 30, 32, 40, 42, 43, 44, 54, 55, 62, 69, 70, 72, 81, 84, 90 and 91 (low-risk types).

2.6. In situ hybridization (ISH)

To confirm the absence of placental HPV by PCR, HPV DNA in situ hybridization (ISH) was conducted on the placentas of eight HPV-positive women whose HPV was detectable using Ventana INFORM HPV probes (Ventana Medical Systems Inc., Tucson, AZ, USA). For ISH, 6 μm-thick sections were cut from formalin-fixed and paraffin-embedded placental samples. The sections were hybridized with HPV DNA probe cocktails after proteolytic treatment. The Family 6 probe cocktail has an affinity for HPV genotypes 6 and 11, and the Family 16 probe cocktail has an affinity for HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66. Placental cells harbouring HPV DNA were identified by haematoxylin-eosin staining, using the HPV genome in SiHa cells as the positive control.

2.7. Statistical analysis

To identify the factors associated with HPV infection in pregnant women and neonates, participants were assigned into one of two groups according to the presence or absence of HPV. Continuous and discrete variables were compared using Student's *t*-test and Chi-squared test, respectively. A p-value <0.05 was considered statistically significant with the use of a two-sided test.

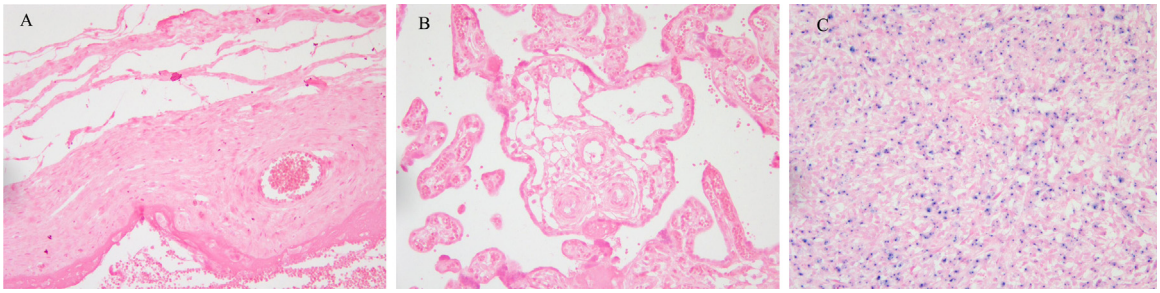

Fig. 1. In situ hybridization of human papillomavirus (HPV) DNA in placental sections. (A) Chorionic plate, (B) Chorionic villi and (C) SiHa cells, as a positive control. The positive control was hybridized with HPV DNA probes, and blue colour indicates the presence of HPV DNA. Panels A and B show an absence of HPV DNA. H&E stain X200.