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Maternal human papillomavirus (HPV) infection and its possible relationship with neonatal prematurity

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Although human papilloma virus (HPV) infection is considered a sexually transmitted infection, it can also be transmitted by non-sexual routes including perinatal transmission, autoinoculation and heteroinoculation and, possibly, indirect transmission via fomites.[1–3] Acquisition of maternal genital HPV infection by infants at birth has been proposed by several researchers.[2,4,5] Newborn babies are exposed to maternal cervical HPV infection which persists for at least six months.[5] Neonatal infections are predominantly of HPV types 16 and 18 and persist in the neonatal genital area as well as in their oral cavity. In a study by Cason *et al*.[5] the transmission rate of infection from HPV-positive mothers to their infants 24 hours after delivery was approximately 73%. The concordance of different HPV types detected in newborn babies and their mothers also indicates perinatal mother-to-infant HPV transmission.[2]

To date, no published data are available concerning the possible relationship between maternal genital HPV infection and their children's antenatal, perinatal or post-natal history. It remains unclear how frequently perinatal HPV infection progresses to clinical lesions, whether they be genital, laryngeal or oral.[2] Moreover, there appear to be no data regarding the possible relationship between genital maternal HPV infection and neonatal prematurity. Factors that can cause neonatal prematurity include maternal chronic infection (group B streptococcus, *Listeria monocytogenes, Ureoplasma urealyticum, Mycoplasma hominis, Trichomonas vaginalis, Gardnerella vaginalis, Bacteroides* spp.).[6]

Over a four-year period (2002–2006), cervical samples were obtained from 276 mothers after the birth of their first child and tested for the presence of HPV using the polymerase chain reaction (PCR) technique. Women with multiple births were not enrolled in the study. The sample included 146 mothers with a normal Papanicolaou smear, 78 mothers with low-grade squamous intraepithelial lesions (SILs), 45 mothers with high-grade SILs and seven with cervical cancer. Genomic and viral DNA was extracted from all collected tissues or smears and stored at –20°C.

Virus detection was performed using a PCR technique as described previously.[7] Briefly, the extracted DNA (1 μL) of each cervical sample was amplified in a total volume of 30 μL

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Table 1. Primers and PCR amplification conditions used for HPV detection and typing.

Gene	Primer	PCR profile
HPV GP5/GP6 (DNA)	5-TTTGTTACTGTGGTAGATAC-3 (GP5)	94˚C, 50 sec (denaturation)
	5-GAAAAATAAACTGTAAATCA-3 (GP6)	52˚C, 45 sec (annealing)
		72˚C, 45 sec (extension)
HPV 16 (DNA)	5-CTGCAAGCAACAGTTACTGCGACG-3 (S)	94˚C, 45 sec (denaturation)
	5-CATACATCGACCGGTCCACC-3 (A)	56˚C, 45 sec (annealing)
		72˚C, 50 sec (extension)
HPV 18 (DNA)	5-AAACTAACTAACACTGGGTTATACA-3 (S)	94˚C, 45 sec (denaturation)
	5-ATGGCACTGGCCTCTATAGT-3 (A)	56˚C, 45 sec (annealing)
		72˚C, 50 sec (extension)
HPV 33 (DNA)	5-AACAGTTAAAAAACCTTTAAA-3 (S)	94˚C, 45 sec (denaturation)
	5-AGTTTCTCTACGTCGGGACCTC-3 (A)	56˚C, 45 sec (annealing)
		72˚C, 50 sec (extension)
HPV 11 (DNA)	5-TGTGTGGCGAGACAACTTTCCCTT-3 (S)	94˚C, 45 sec (denaturation)
	5-TGGTTATTTAGTTTTATGAAGCGTGCCTTTCCC-3 (A)	56˚C, 45 sec (annealing)
		72˚C, 50 sec (extension)
β2-m (DNA)	5-TCCAACATCAACATCCCGGT-3 (S)	94˚C, 50 sec (denaturation)
	5-TCCCCCAAATTCTAAGCAGA-3 (A	52˚C, 45 sec (annealing)
		72˚C, 45 sec (extension)

S: sense primer; A: antisense primer.

containing 5 μmol/L 10x PCR reaction buffer (200 mmol/L Tris-HCl [pH 8.4], 500 mmol/L KCl), 1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 0.5 μmol/L each primer and 0.6 units recombinant *Thermus aquaticus* (*Taq*) DNA polymerase (Invitrogen, UK). The presence of amplifiable DNA was verified by performing PCR using primers specific for β2-microglobulin. To distinguish HPV types, separate specific pairs of primers for the high-risk HPV types 16, 18 and 33, and the low-risk HPV 11 were used, giving a different length of amplified DNA. The sequences of all primer sets used and the PCR profile are presented in Table 1.

The extracted DNA (1 μL) from each sample was amplified in a total volume of 20 μL containing 10x PCR reaction buffer (1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 0.5 μmol/L each primer [sense and antisense] and 0.8 unit recombinant *Taq* DNA polymerase [Invitrogen]). The PCR products were analysed on 2% agarose gel and photographed on an ultraviolet (UV) light transilluminator. The PCR product size of HPV 16 was 315 bp; HPV 18, 143 bp; HPV 33, 171 bp; and HPV 11, 236 bp. The PCR assay was performed in a PTC-200 programmable thermal controller (MJ Research, USA). All PCR reactions included appropriate negative controls. Extracted DNA from HeLa cells and the plasmid DNA of HPV 16, 18, 33 and 11 were used as positive controls.

A questionnaire was constructed to obtain details about the mothers and their partners, as well as the antenatal, birth and post-natal history of their children. The questionnaire included 10 open questions regarding i) parental socioeconomic and educational status, ii) parental age, iii) parental smoking status, iv) previous history of HPV infection, v) previous history of genital infection, vi) antenatal history and high-risk delivery factors, vii) duration of neonatal gestation, viii) mode of delivery, ix) post-natal complications, and x) need for intensive neonatal care.

Statistical analysis was performed using SPSS software (SPSS, version 11.5). The χ^2 and *t*-tests were used to compare different clinicopathological parameters with the duration of neonatal gestational or maternal genital HPV status. Statistical significance was set at $P<0.05$. The study was approved by the ethics committee of the University of Crete and all participants gave written informed consent.

One hundred and forty-seven women were positive for HPV. Type 16 infection was detected in 57 (38.8%), HPV 18 in 41 (27.9%), HPV 33 in 17 (11.6%) and HPV 33 in eight (5.4%). HPV 16/18 co-infection was detected in four (2.7%) women, while in 28 (19%) HPV-positive women the type was not identified.

As shown in Table 2, duration of neonatal gestation was significantly lower in children born to HPV-positive mothers compared to those born to HPV-negative mothers (37 weeks *vs*. 39 weeks, $P=0.011$). Similar statistically significant results were observed in the duration of neonatal gestation between HPV 16- or 18-positive and HPV-negative mothers (36 weeks *vs*. 39 weeks, $P=0.005$) and between HPV 11-positive mothers and HPV-negative mothers (38 weeks *vs.* 39 weeks, $P=0.041$). There was no statistically significant difference in the duration of neonatal gestation between HPV 33 and HPV-negative mothers (39 weeks *vs.* 39 weeks, $P=0.853$).

Two hundred and fifty children were delivered after 36 weeks' gestation, and 26 (9.4%) were premature. Maternal HPV infection was detected in 128 (51.2%) children delivered after 36 weeks' gestation. The respective rate of maternal HPV infection was 73.1% among premature neonates born at less than 36 weeks and 83.3% among premature neonates born at less than 32 weeks.

Mean ages among HPV-positive and HPV-negative mothers were 28 years (range: 16–41) and 32 years (range: 18–38), respectively. The sample included 26 (9.4%) mothers of low socioeconomic status and 35 (12.7%) who were poorly

Table 2. Neonatal gestational age and maternal age in relation to maternal HPV infection.

	Children born to HPV-positive mothers (n=147)	Children born to HPV-negative mothers (n=129)
Mean neonatal gestational age (range)	37 (37±4) weeks	39 (39±2) weeks
<28 weeks	0	0
28–30 weeks	2 (1.4%)	1 (0.8%)
31–32 weeks	3 (2.0%)	0
33–34 weeks	4 (2.7%)	1 (0.8%)
35–36 weeks	10 (6.8%)	5 (3.9%)
37–38 weeks	26 (17.7%)	27 (20.9%)
39–40 weeks	95 (64.6%)	88 (68.2%)
41–42 weeks	7 (4.8%)	7 (5.4%)
Mean maternal age at birth (range)	28 (16–41) years	32 (18–38) years

educated. The frequency of HPV infection among women of low socioeconomic status was 84.6%, while the frequency among women of high socioeconomic status was 51% (P=0.007). Infection was also detected more frequently among women who had received less than 12 years' education than among more highly educated women (97.1% *vs.* 49.4%, P=0.001). In the total sample, the frequency of maternal smoking was 56.1% (HPV-positive women: 57%, HPV-negative women: 55.6% [P=0.372]).

Among 26 premature neonates, maternal infection was considered to be the causative risk factor for prematurity in 15 cases (57.7%), congenital defects were observed in two cases (7.7%), while in seven (34.6%) cases the cause of prematurity was not identified. Interestingly, no statistical relationship was observed between duration of gestation and maternal age (P=0.675), maternal smoking (P=0.113), socioeconomic (P=0.671) or educational status (P=0.892).

To the authors' knowledge, this is the first report to find a relationship between maternal HPV status and duration of gestation; however, the mechanism by which HPV infection is related to prematurity remains unclear. It is well known that HPV infects epithelial cells through abrasion of the cervical mucosa, where it can exist as a long-term latent infection that can reactivate or persist.[8] However, there is no laboratory evidence to support the possible direct impact of HPV infection on neonatal gestation.

One possible explanation is that HPV is an indirect index of the presence of other sexually transmitted infections related to neonatal prematurity.[9,10] It has been demonstrated that persistence of maternal HPV infection is related to co-infection with other sexually transmitted agents such as HIV, herpes simplex virus type 2 and *Chlamydia trachomatis*.[9,10] Overt or asymptomatic bacterial infection including chronic *C. trachomatis* infection of the amniotic fluid and membranes may initiate preterm labour.[7]

Bacterial products can stimulate the production of local inflammatory mediators (e.g., interleukin 6 and prostaglandins), which may induce premature uterine contractions or a local inflammatory response with focal membrane rupture. However, an indirect role for HPV infection as an index of the presence of other sexually transmitted infections related to neonatal prematurity remains to be elucidated.[9,10]

Socioeconomic status, maternal age and smoking status are also related to neonatal prematurity,[6] all of which have been implicated in the persistence of cervical HPV infection.[9,10] Interestingly, in the present study, maternal age was lower in HPV-positive mothers than in HPV-negative mothers. Moreover, detection of HPV infection was related to lower socioeconomic and educational status, but duration of gestation was not related to maternal age, socioeconomic and educational status.

In summary, the findings presented here suggest that the presence of HPV could be a marker of neonatal prematurity; however, this is a preliminary study and further work on a greater number of subjects is required to identify possible confounding variables such as maternal infection or the presence of congenital defects. □

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