Evidence of Human Papillomavirus in the Placenta

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Human papillomavirus (HPV) is an epitheliotropic virus typically infecting keratinocytes but also possibly epithelial trophoblastic placental cells. In the present study, we set out to investigate whether HPV can be recovered from transabdominally obtained placental cells to avoid any confounding contamination by HPV-infected cervical cells. Thirty-five placental samples from women undergoing transabdominal chorionic villous sampling were analyzed, and we detected HPV-16 and HPV-62 in 2 placentas. This study suggests that HPV infection of the placenta can occur early in pregnancy. The overall clinical implication of these results remains to be elucidated.

Infection of cervical cells with a high-risk human papillomavirus (HPV) is a prerequisite in the development of cervical cancer. Furthermore, HPV has been reported to infect placental material and to be associated with spontaneous abortions in the first and second trimester of gestation, with infection rates varying from 7% [1] to 50%–70% [2–4]. Nevertheless, these previous results could not exclude a possible contamination of the placental cells with HPV DNA from the cervix. In vitro experiments suggested several molecular mechanisms providing plausible explanations for the association between placental HPV infection and the adverse outcome of pregnancy. Ectopic HPV-16 E5, E6, and/or E7 expression in trophoblastic cell lines has been associated with changes in viability, reduced adhesion, enhanced migration, and enhanced invasion of these cells [2, 5, 6]. Besides, the HPV-16 early promoter can be constitutively activated in several trophoblastic cell lines, which could be partly attributed to secreted progesterone, hence emphasizing the hormonal regulation of the HPV gene expression regulation during pregnancy (C. Weyn, J. Rasschaert, J.-M. Vanderwinden, Y. Englert, and V. Fontaine, submitted).

Other studies on the vertical transmission of HPVs have often been hampered by the possible contamination of the placenta with cervical cells from an infected birth canal. To circumvent this bias, we analyzed transabdominally obtained placental samples to examine strictly intrauterine HPV placental infection.

MATERIALS AND METHODS

From November 2008 until January 2010, 35 women gave their informed consent to use residual material from abdominal chorionic villous sampling after cytogenetical examinations for the HPV detection study by our laboratory. The gestational age of the sampling was between the 11th and the 13th gestational week. This study was approved by the local ethical committees of the different participating hospitals (reference P2008/222).

All samples were treated upon arrival. DNA was extracted and eluted in 50 μL using the High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit, according to the manufacturer's protocol (Roche Diagnostics). DNA quality was verified by PCR on the β-globin gene with the GH20 and PC04 primers, as described elsewhere [7].

The extracted DNA from placental tissue was used in 3 different consensus PCR reactions in 2 different regions of the viral genome. The L1 region was amplified using 2 sensitive nested PCR strategies, both based on a general PGMY09/11 PCR, followed by a second nested PCR with either the GP5+/6+ or the SPF10 primers, performed as described elsewhere [8]. The pU PCR, amplifying the E6/E7 region, was performed in parallel, using 50 pmol/L pU-1-M-L forward primer, 25 pmol/L of the pU-2-R and 25 pmol/L of the pU-2-R-N reverse primers, as described elsewhere [9]. This PCR was shown to amplify HPV-16, -18, -26, -30, -31, -33, -35, -39, -45, -51, -52, -58, -59, -66, -68, -73, -85 [9,] and not HPV-34, -53, -56, -82 (our unpublished results). Negative controls were included in every PCR reaction to rule out possible laboratory cross-contamination.

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HPV genotypes were determined with the INNO-LiPA Genotyping v2 kit (Innogenetics, Ghent, Belgium). This reverse hybridization line probe assay relies on the amplification of a 65 bp fragment in the L1 region with the SPF10 biotinylated primers, amplifying 23 HR- and LR-HPV types (HPV-6, -11, -16, -18, -31, -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -66, -68, -70, -74), followed by streptavidin conjugation and substrate signal amplification [10]. HPV pU and GP5+/6+ PCR products were purified using a GFX column (Amersham) according to the manufacturer's instructions. Direct DNA sequence analysis was performed using a capillary sequencer (ABI Prism 3130).

RESULTS

The residual trophoblastic placental cells used for HPV detection were obtained from 35 placental samples of women undergoing chorionic villous sampling (CVS) for different clinical indications, mainly being patients with a high risk for chromosomal abnormalities. HPV DNA was detected in 2 out of 35 placental samples. The first sample contained HPV-16, classified as a high-risk HPV genotype, as identified by the INNO-LiPA genotyping method. This genotype could be confirmed upon sequencing of the E6/E7 PCR product. The combination of 2 PCR reactions in different regions of the viral genome leaves no doubt about the presence of a true HPV-16 infection. The second HPV-positive sample harbored HPV-62, classified as an HPV genotype of undetermined risk, as characterized through sequencing of the GP5+/6+ amplified fragment. This sample was negative after pU PCR, which is not surprising considering the low amount of available material and the low sensitivity of the primers to recognize the HPV-62 E6/E7 DNA. Accordingly, the INNO-LiPA genotyping method after SPF10 PCR was unable to identify HPV-62, since this type is not included in the test. The results are summarized in Table 1. It is noteworthy that HPV-62 was never detected before in our laboratory, hence further ruling out possible laboratory cross-contamination.

DISCUSSION

We demonstrated the presence of HPV in 2 out of 35 transabdominally obtained placental samples, although we cannot exclude the actual HPV prevalence being higher, given that the analysis was restricted by limited amounts of placental material available, as recommended by the ethical committee.

Table 1. HPV) Polymerase Chain Reaction Results Obtained for the HPV-Positive Chorionic Villous Sampling

Results	Sample 1	Sample 2
L1 region:PGMY09/11 and GP5+/6+ (Sequencing)	HPV-16	HPV-62
L1 region:PGMY09/11 and SPF10 (INNO-LiPA)	HPV-16	undefined
E6/E7 region:pU (Sequencing)	HPV-16	undefined

NOTE. The genotyping method is mentioned in brackets.

Since HPV-16 is often detected in cervical samples, we propose that placental infection might occur either through an ascending infection from the cervix or via infected sperm at fecundation. Indirect evidence supporting this first theory was postulated by Hermonat and colleagues [3] who observed a higher spontaneous abortion rate in women with cervical HPV infection compared with HPV-negative women (60% vs 20%). It is noteworthy that when retrospectively inquiring for the HPV cervical status from the 2 patients having an HPV positive CVS, the patient harboring an HPV-16 positive placenta had a HPV-negative cervical smear 9 months before and 14 months after the puncture. This information might be difficult to interpret, as the time of the puncture and of the cervical anatomopathological analyses did not coincide. This could, however, also suggest a placental HPV infection via infected sperm [11]. A hematogenous infection seems less likely but cannot be excluded. Our results are in concordance with previous studies suggesting vertical transmission of both α- and β-HPVs [12, 13]. On the other hand, our results are in discordance with one study reporting the absence of HPV DNA in 147 abdominally obtained placental samples, possibly due to the use of a less sensitive PCR strategy [14]. In our study, we optimized all PCR reactions in order to obtain maximum sensitivity through adaptation of annealing temperature, ramp speed, and Mg^{2+} concentration [8].

The etiological association between HPV placental infection and a possible adverse outcome on pregnancy was not examined in our study and could therefore not be underlined. Based on the aforementioned in vitro studies, one could hypothesize that placental HPV infection could play a role in spontaneous pregnancy loss as a consequence of impaired trophoblast implantation and placentation. We could speculate that a viral infection of the placenta could lead to fetal loss even before the 11^{th} week of gestation, an event that could not be monitored by our laboratory since we only obtained placental material from the 11^{th} gestational week onward.

Vertical HPV transmission could possibly be prevented by vaccination, although the clinical impact of placental HPV infection will require further study. In conclusion, HPV-16 and HPV-62 were detected in 2 out of 35 transabdominally obtained placental punctures. Additional larger studies will be needed to assess HPV type distribution and prevalence in the placenta.

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