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Placental human papillomavirus infections and adverse pregnancy outcomes

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ABSTRACT

Introduction: Knowledge on prevalence and association of human papillomavirus (HPV) in third trimester placentae and adverse pregnancy outcomes is limited. We investigated the prevalence of placental HPV at delivery, explored urine HPV characteristics associated with placental HPV and whether placental HPV increased the risk adverse pregnancy outcomes.

Methods: Pregnant women were enrolled in the Scandinavian PreventADALL mother-child cohort study at midgestation. Human papillomavirus genotyping was performed on placental biopsies collected at delivery (n = 587) and first-void urine at midgestation and delivery (n = 556). Maternal characteristics were collected by questionnaires at gestational week 18 and 34. Adverse pregnancy outcomes were registered from chart data including hypertensive disorders of pregnancy, gestational diabetes mellitus and newborns small for gestational age. Uni- and multivariable regression models were used to investigate associations.

Results: Placental HPV was detected in 18/587 (3 %). Twenty-eight genotypes were identified among the 214/556 (38 %) with midgestational urine HPV. Seventeen of the 18 women with placental HPV were midgestational HPV positive with 89 % genotype concordance. Midgestational high-risk-(HR)-HPV and high viral loads of Any- or HR-HPV were associated with placental HPV. Persisting HPV infection from midgestation to delivery was not associated with placental HPV. Adverse pregnancy outcomes were seen in 2/556 (0.4 %) of women with placental HPV.

Discussion: In this general cohort of pregnant women, the prevalence of placental HPV was 3 %, and midgestational urinary HPV 38 %. High HPV viral load increased the risk for placental HPV infections. We observed no increased risk for adverse pregnancy outcomes in women with placental HPV.

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Abbreviations

BMI	Body Mass Index (kg/m^2)
GW	Gestational week
GDM	Gestational diabetes mellitus
HDP	Hypertensive disorders of pregnancy
HPV	Human papillomavirus
HR-HPV	High-risk human papillomavirus
PCR	polymerase chain reaction
SGA	newborns small for gestational age

1. Informed consent

Written informed consent was obtained from all women upon enrollment with the option to withdraw from the study with no reason given.

2. Introduction

Human papillomavirus (HPV) is a small double stranded DNA virus. Human papillomavirus infections are the most common sexually transmitted disease, and most prevalent during the reproductive years of women [1,2]. The role of HPV in cervical carcinogenesis is well founded where high viral loads and persisting HPV infections play a crucial role, whereas the role of HPV infections during pregnancy is less clear [1–6].

Human papillomavirus has been detected in placentae from generally healthy women [7–14] [5,15-21], with third trimester placental HPV prevalence ranging from 0 to 75 % [5,7,13,15–17,19,22,23]. It has also been shown that HPV can be cultivated in placental tissue (trophoblast cells) and carry out complete replication cycles [7–12]. In addition to trophoblast cells, other cells of the placenta have been shown to harbor HPV, such as Hofbauer cells and parts of the decidua [15,17]. The HPV infection may affect viability and adhesion properties as well as migration and invasion of the trophoblasts [7,9,11]. However, there is limited information whether HPV characteristics, such as viral load and persistence, predict transmission of HPV from the genital tract to the placenta [5].

Given the crucial role of trophoblast cells in the normal development and function of the placenta, placental HPV infection might possibly increase the risk of placental dysfunction syndromes such as hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM) and newborns small for gestational age (SGA) [24–26].

The primary aim of this study was therefore to assess the prevalence of placental HPV at delivery overall and by maternal characteristics in a large sample of generally healthy pregnant women. Secondarily, we aimed to explore midgestational urine HPV characteristics associated with placental HPV at delivery. Thirdly, we aimed to explore whether placental HPV at delivery was associated with adverse pregnancy outcomes.

3. Methods

3.1. Study design and study population

Women for this study were recruited from the Preventing Atopic Dermatitis and Allergies in children (PreventADALL) study, a multicenter prospective population-based mother-child cohort study [27]. Recruitment of women occurred from December 2014 to October 2016. Pregnant women who provided first-void urine samples at the time of recruitment, gestational week (GW) 16–22, and at the time of delivery, and where placenta at delivery was available, were eligible for the current study. Extensive socio-demographic data was collected using electronic (e−) questionnaires at GW 18 and 34. Medical records were reviewed for relevant and comprehensive medical information and pregnancy outcomes. Of the 2697 women included in the PreventADALL study, three women were excluded as they withdrew from the study. Four women were included with two separate pregnancies, if both pregnancies had valid HPV urine result (i.e. valid human housekeeping gene internal DNA control and/or HPV positive), the first pregnancy was selected. In addition to this, twelve twin pregnancies were excluded. For the primary aim we excluded pregnancies with missing placenta at delivery, yielding a total of 587 placentae available for biopsies. For the aims 2 and 3 we excluded women with missing or invalid urine HPV samples (invalid human housekeeping gene internal DNA result and HPV negative) at midgestation (n = 31) with a total of 556 women with valid urine HPV samples and placentae available for biopsies. Persistence analysis was performed in women with HPV positive result at midgestation. All of them had a valid HPV result at delivery.

For the sensitivity analysis for aims 2 and 3, women with a negative urine HPV result at midgestation (n = 342) were excluded, yielding 214 women (Fig. 1).

3.2. Human papillomavirus sample collection and detection

Pregnant women were asked to provide first-void urine samples at the time of enrollment (GW 16–22) and delivery. Placenta biopsies were collected immediately after delivery. A detailed description of urine sample handling has been described previously [3]. Briefly, first-void urine was collected and immediately placed in a refrigerator (4 °C) for maximum of 30 h before it was stored at −80 °C until analysis. Detection and genotyping of HPV in the urine was performed using Seegene Anyplex II HPV28 detection polymerase chain reaction (PCR) assay (Seegene Inc, Seoul, South Korea) [3].

Placenta biopsies were taken by either the study team or by the

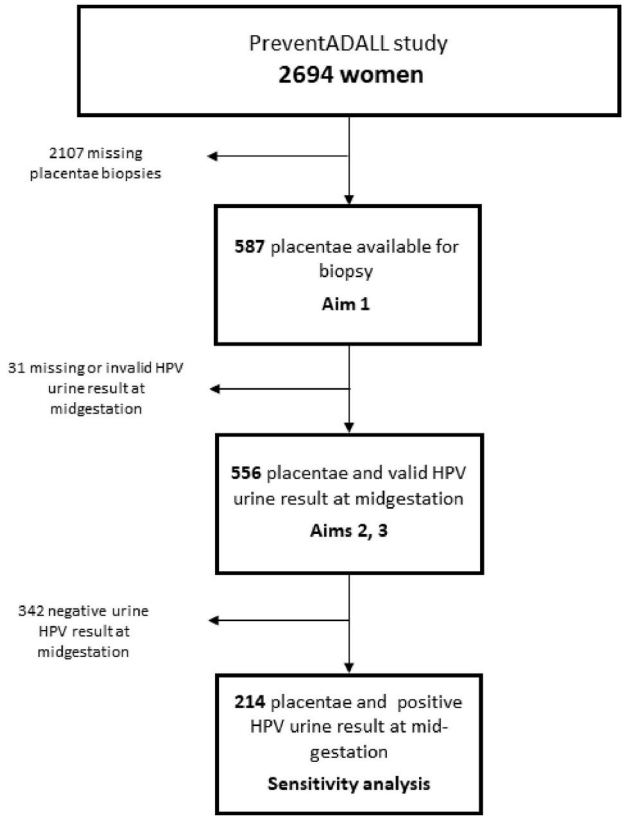

Fig. 1. Flow chart of the present study population from the PreventADALL general population mother-child cohort where 2697 women initially were enrolled. Three women later withdrew together with their offspring and data, leaving 2694 included women. Abbreviations: HPV- human papillomavirus.

midwife/nurse assistant involved in the delivery. Detailed protocols were followed to ensure high quality biological samples. Three full thickness punch biopsies of 5 mm diameter from peripheral, mid, and central portion of the placenta were taken to obtain representable samples containing placental villi and or decidual cells, maximizing the chance for detecting HPV infections. The three samples were placed together in 2 mL tubes containing 1.5 mL RNALater® (Life Technologies, Carlsbad, California, USA) and stored at 4 °C for maximum 30 h and then at −80 °C until analysis [28,29]. To extract DNA from placental cells, a specific Tissue based DNA extraction kit was chosen, that makes use of magnetic beads that bind to both short and long DNA molecules and is suitable for a large range of applications. Samples from all three placental biopsies were used for DNA extraction, using Maxwell®RSC Tissue DNA kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocol detection, and genotyping of 19 HPV types, as previously described, using a duplex In House Real-Time PCR protocol with published primer and probes for HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and in-house designed primer and probes for HPV types 42, 66, 68, 73 and 90. Seegene Anyplex II HPV28 PCR assay was also used for placental biopsies to detect 28 HPV genotypes, as previously described [3]. Appendix 1 in Supplementary material details the methods used. Supplementary Table 1 displays the complete list of HPV genotypes detected by In House Real-Time PCR on Quantstudio™ 7 Flex Real-Time PCR System (QS7) and Seegene Anyplex II HPV28 PCR assay, stratified according to the malignant potential of HPV as described by International Agency for Research on Cancer [30].

3.3. Exposure and outcomes

Exposures and outcomes are defined in Table 1.

Placental HPV: was defined as Any-HPV infection (yes, no) included women with any of the 29 detectable genotypes by In House Real-Time PCR assay and by Seegene Anyplex II HPV28 PCR assay, in placenta at delivery.

Midgestational urine HPV: was defined as Any-HPV (yes, no) included women with any of the 28 detectable genotypes in their urine detected at midgestation and High-Risk (HR)-HPV (yes, no) included women with any of the 12 H R-HPV genotypes detected (Supplementary Table 1).

High viral load in midgestational urine (yes, no): was defined as no infection/low viral load versus high viral load. Viral load was defined according to results generated from Seegene Anyplex II HPV28 PCR assay with + corresponding to low viral load and ++/+++ to high viral load [3].

Persistence of HPV infection during pregnancy (yes, no): was defined as no genotype specific persistence of HPV infection found in urine samples at midgestation and delivery versus genotype specific persistence during pregnancy. The likelihood of a pregnant woman engaging with a new sexual partner during the latter part of her pregnancy and thus contracting a new HPV infection of same genotype as found at enrollment, was regarded as improbable.

Adverse pregnancy outcomes: Women were defined as having placental dysfunction syndromes (yes, no) if they had any of the following disorders: hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM) or newborns small for gestational age (SGA). Definitions for the abovementioned adverse pregnancy outcomes are shown in Supplementary Table 2.

Table 1
Definition of exposures and outcomes for Aims 2 and 3 for the current study.

	Exposure	Outcome
Aim 2	o Midgestational HR-HPV[a] infection (yes, no) o High Any-HPV[b] viral load in urine at midgestation (no infection/low viral load, high viral load) o HR-HPV[a] viral load in urine at midgestation (no infection/low viral load, high viral load) o Persisting HPV in urine from midgesation to delivery.	o Any-HPV[c] in placenta (yes, no)
Aim 3	o Any-HPV[c] in placenta (yes, no)	o Placental dysfunction syndromes[d] (yes, no)

[a] HR-HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.
[b] Any-HPV in urine 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61.
[c] Any-HPV in placenta 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61, 90.
[d] Placental dysfunction syndromes consisting of hypertensive disorders of pregnancy, gestational diabetes mellitus, newborns small for gestational age. Women were defined as having a placental dysfunction syndrome if they had any of the abovementioned disorders.

3.4. Statistical analyses

Categorical variables are presented with numbers and percentages and continuous variables as means with standard deviations or medians with minimum and maximum values.

For the primary aim (N = 587), descriptive analysis was performed.

For aim 2, we performed univariable and multivariable logistic regression or exact logistic regression models with placental HPV infection as outcome and 1) midgestational HR-HPV infection (N = 556) 2) high midgestational Any-HPV or HR-HPV viral load (N = 556) and 3) persisting Any-HPV or HR-HPV infections (N = 178/N = 114) as exposures. For the multivariable logistic/exact logistic regression models we adjusted for duration of preconception relation with child's father (<3 years, 3–5 years and >5 years) according to Direct Cyclical Graphs (DAG) using Dagitty.net [31] (Supplementary Figure 1). The covariate duration of preconception relation to child's father is an aggregated variable from the original variable (duration <12 months, 1–2 years, 3–5 years and >5 years). We coded missing values as a separate category in order not to lose any women in the models. Exact logistic regression models were used in cases of cells with n < 5. In women who had both positive urine and placental HPV infection, we compared concordance of genotypes identified.

For aim 3 (N = 556), we performed a univariable exact logistic regression model with placental dysfunction syndromes as outcomes and placental HPV infection as exposure. Multivariable regression analysis was not performed due to small number of women with placental dysfunction syndromes.

To reduce residual confounding, we performed a sensitivity analysis of aim 2 and 3 including only women with positive midgestational urine HPV infections. Sensitivity analysis was not performed for persisting infections as the definition of a persisting infection is having a positive HPV infection at midgestation and at delivery thus only HPV positive women were included.

Placental HPV results from the In House Real-Time PCR assay and Seegene Anyplex II HPV28 PCR assay were made into a new variable by aggregating results, making sure not to include duplicates. There was full concordance between the In House Real-Time PCR assay and Seegene Anyplex II HPV28 PCR assay results, with no conflicting results. It must be noted that Seegene Anyplex II HPV28 PCR assay does not detect HPV 90, which is detected by the In House Real-Time PCR assay.

All statistical analyses were performed using Stata version 16 (StataCorp LLC, Texas). A p-value <0.05 was considered statistically significant.

4. Results

4.1. Study sample

In women with available placenta for biopsies (N = 587), the mean age at enrollment was 31.8 years and the median pre-pregnancy BMI 24.5 (Table 2). Most women were married or cohabitants (98 %) and

Table 2
Baseline characteristics for participants (N = 587), overall and by HPV placental infection.

	Total N = 587	Placenta Biopsy Any-HPV[a] positive N = 18	Placenta biopsy Any-HPV[a] negative N = 569
Maternal Age, yr. Mean (SD)	31.8 (4.6)	31.0 (4.0)	31.8 (4.6)
Maternal pre-conception BMI. Median (min-max)	24.5 (17.8–48.2)	24.9 (21.9–34.1)	24.5 (17.8–48.2)
Marital Status. N (%)			
Married/ cohabitants	502 (98 %)	15 (93 %)	487 (98 %)
Singe/Divorced/ Other	11 (2 %)	1 (6 %)	10 (2 %)
Maternal education. N (%)			
Higher/PhD/> 4 years	248 (49 %)	9 (56 %)	239 (48 %)
Higher education ≤4 years	186 (37 %)	4 (25 %)	182 (37 %)
Preliminary/high school only	75 (14 %)	3 (18 %)	72 (15 %)
Duration of pre-conception relation with child's father, yr. N (%)			
>5	319 (63 %)	4 (29 %)	315 (64 %)
3-5	108 (21 %)	2 (14 %)	106 (21 %)
<3 years	80 (16 %)	8 (57 %)	72 (15 %)
Nicotine use during pregnancy. N (%)			
No	447 (87 %)	15 (94 %)	432 (87 %)
Yes	66 (13 %)	1 (6 %)	65 (13 %)
Alcohol consumption during pregnancy. N (%)			
No	340 (67 %)	11 (73 %)	329 (66 %)
Yes	171 (33 %)	4 (27 %)	167 (34 %)
Number of previous deliveries (Parity). N (%)			
0	338 (58 %)	14 (78 %)	324 (57 %)
≥1	248 (42 %)	4 (22 %)	244 (43 %)
Delivery mode. N (%)			
Vaginal/Vaginal-instrumental	488 (83 %)	18 (100 %)	470 (83 %)
C-section	99 (17 %)	0	99 (17 %)
Fetal sex. N (%)			
Male	304 (52 %)	7 (39 %)	297 (52 %)
Female	283 (48 %)	11 (61 %)	272 (48 %)
Gestational age at birth, weeks. Mean, (SD)	40.2 (1.4)	39.9 (1.1)	40.2 (1.4)
Newborn weight, grams. Mean, (SD)	3606 (466.7)	3390 (275.2)	3613 (470.0)

Missings: numbers and proportions of categorical variables are based on available information. Missing information in continuous variables were; maternal age, gestational age and, newborn weight-0 missing, pre-conception BMI-1 missing.
Abbreviations: BMI-body mass index, HPV-human papillomavirus, max-maximum, min-minimum, SD−standard deviation, yr-years.
[a] Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61,90.

had higher education (85 %). The mean gestational age at birth was 40.2 weeks and 12 women gave birth prematurely (GW 32–36). The mean newborn birth weight was 3606 g (Table 2).

4.2. Placental human papillomavirus at delivery

Human papillomavirus was detected in placental biopsies in 18/587 (3 %) women. The most common genotypes detected were Low-Risk (LR)-HPV42, 4/18 (22 %) and HR-HPV31 and HR-HPV51, both with 3/18 (17 %). In total 10 genotypes were detected, HPV16, 31, 35, 42, 45, 51, 58, 61, 69, and 90. Only one woman had multiple HPV genotypes in the placental biopsy, HPV35 and HPV42 (Fig. 2).

Maternal characteristics of women with and without placental HPV infections did not differ greatly (Table 2). None of the women who gave birth prematurely had placental HPV infection. Women with shorter preconception relation to child's father (<3 years, n = 80) more often had a positive placental HPV result (8/80, 10 %) compared to women with a preconception relation to child's father of >5 years (4/319 1 %) (Table 2). There were no major differences in baseline characteristics between women included in the study (N = 556) and non-included women (N = 2138, Supplementary Table 3).

4.3. Genital human papillomavirus and placental HPV infection

The prevalence of midgestational Any-HPV in urine was 214/556 (38 %) and the prevalence of placental HPV in women with positive midgestational HPV urine result was 17/214 (8 %), meaning that seventeen of the 18 (94 %) pregnancies with placental HPV infection also had a positive HPV urine result at midgestation. The genotype concordance between midgestational urine HPV and placental HPV was 16/17 (89 %). Detection of urine HPV at delivery and placental HPV was 11/18 (61 %) and 4/18 women with placental HPV had prior midgestational HPV positive urine result but were HPV negative at delivery. One woman with positive HPV urine at delivery and in the placenta had a negative midgestational urine HPV result, but with no genotype concordance between urine at delivery and placental HPV (Fig. 2).

High-Risk HPV infection was detected in midgestational urine in 15/18 (83 %) of women with a positive placental HPV result, regardless of the HPV type detected in placenta. High viral load of Any-HPV in midgestational urine was found in 15/18 (83 %) of positive placental HPV and high viral loads of HR-HPV in midgestational urine in 10/18 (55 %) where 9/18 (50 %) placental HPV infections were with corresponding HR-HPVs. We observed that 11/18 (61 %) women with placental HPV infections had multiple HPV infections at midgestation.

Midgestational HR-HPV in urine was significantly associated with placental HPV infection (adjusted (a)OR = 13.1 (95%CI 3.53–73.21) compared to no-HR-HPV women; $p < 0.001$). High viral load of Any-HPV and high viral load of HR-HPV in midgestational urine were associated with placental HPV infection (aOR = 14.7 (95%CI 3.90–82.22)); $p < 0.001$ and (aOR = 8.5 (95%CI 2.77–26.53); $p < 0.001$, respectively) (Table 3).

There were no statistically significant associations between persisting Any-HPV/HR-HPV in urine from midgestation to delivery and placental HPV at delivery (aOR = 1.93 (95%CI 0.52–8.96); p = 0.433 and (aOR = 1.2 (95%CI 0.20–8.31); p = 1.000, respectively) (Table 3).

4.4. Placental human papillomavirus infections and placental dysfunction syndromes

Placental dysfunction syndromes was found in 122/556 (22 %) of the women with available placentae for biopsy. Out of the 122 women with adverse pregnancy outcomes, Any-HPV placental infection was detected in only two pregnancies with placental dysfunction syndromes, one with HDP and one with SGA. No association was observed between placental HPV and the placental dysfunction syndromes (OR = 0.4 (95%CI 0.5–1.90); p = 0.411) (Table 4).

4.5. Sensitivity analyses

In women with positive HPV urine result at midgestation (N = 214), HR-HPV was no longer significantly associated with placental HPV infection (aOR = 4.2 (95%CI 0.94–39.83); p = 0.06). Regarding high viral loads infections in the 214 women with positive HPV at midgestation only, results were similar to the analyses in the 556 women included in the present study population (Supplementary Table 4). Likewise, in the sensitivity analysis of aim 3, results were similar to the results to the main analysis but now only one woman was diagnosed with an adverse pregnancy outcome (Supplementary Table 5).

5. Discussion

We found that the overall prevalence of placental HPV infections was

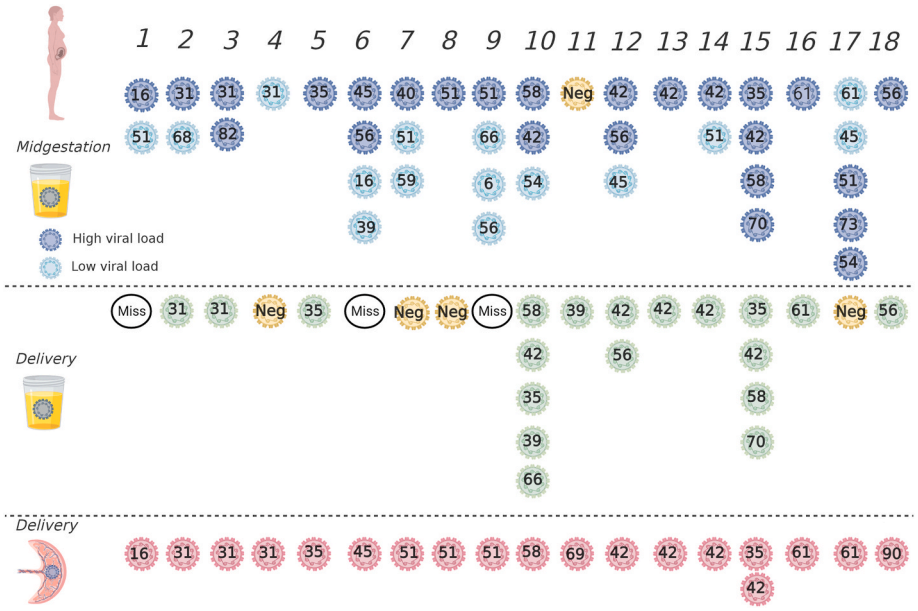

Fig. 2. Graphical illustration of urine HPV and placental HPV infection in the 18 women with positive HPV result in the placenta.

Table 3
Association between urine HPV infection during pregnancy and placental HPV infections (N = 556 for urine HPV at midgestation and for persistent infection from midgestation to delivery).

	Total N = 556	Placenta Biopsy Any-HPV[a] positive N = 18	Placenta biopsy Any-HPV[a] negative N = 538	Unadjusted OR (95 % CI)	p-value	Adjusted[b] OR (95 % CI)	p-value
Urine HPV at midgestation N = 556							
HR-HPV[c]. N (%)					**<0.001**[d]		**<0.001**[d]
No	419 (75 %)	3 (17 %)	416 (77 %)	1			
Yes	137 (27 %)	15 (83 %)	122 (23 %)	16.9 (4.69–92.78)		13.1 (3.53–73.21)	
High Any-HPV[a] viral load. N (%)					**<0.001**[d]		**<0.001**[d]
No infection/low viral load	433 (78 %)	3 (17 %)	430 (80 %)	1			
High viral load	123 (22 %)	15 (83 %)	108 (20 %)	19.8 (5.46–108.40)		14.7 (3.90–82.22)	
High HR-HPV[c] viral load. N (%)					**<0.001**[e]		**<0.001**[e]
No infection/low viral load	492 (88 %)	8 (44 %)	484 (90 %)	1			
High viral load	64 (12 %)	10 (55 %)	54 (10 %)	11.2 (4.24–29.60)		8.5 (2.77-26-53)	
Persistent infection from midgestation to delivery							
	Any-HPV positive at midgestation N=178	**Placenta Biopsy Any-HPV[a] positive N=14**	**Placenta biopsy Any-HPV[a] negative N=164**	**Unadjusted OR (95 % CI)**	**p-value**	**Adjusted[b] OR (95 % CI)**	**p-value**
Persisting urine Any-HPV[a] at delivery N (%)					0.409[d]		0.433[d]
No	76 (43 %)	4 (29 %)	72 (44 %)	1			
Yes	102 (57 %)	10 (71 %)	92 (56 %)	1.9 (0.53–8.87)		1.9 (0.52–8.96)	
	HR-HPV positive at midgestation N=114	**Placenta Biopsy HR-HPV[c] positive N=8**	**Placenta biopsy HR-HPV[c] negative N=106**			**Adjusted[b] OR (95 % CI)**	**p-value**
Persisting urine HR-HPV[c] at delivery N (%)					1.000[d]		1.000[d]
No	50 (44 %)	3 (38 %)	47 (44 %)	1			
Yes	64 (56 %)	5 (62 %)	59 (56 %)	1.3 (0.24–8.97)		1.2 (0.20–8.31)	

Abbreviations: CI-confidence interval, HPV-Human papillomavirus, HR-HPV-High-Risk Human papillomavirus, *N*-total number, OR-odds ratio.

[a] Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61,90 (HPV90 is not included in Any-HPV in urine).

[b] Adjusted for duration of preconception relation with child's father.

[c] HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.

[d] Exact logistic regression model.

[e] Logistic regression model.

Table 4
Association between placental HPV and placental dysfunction syndromes (N = 556).

	Total N = 556	Placenta Biopsy Any-HPV[a] positive N = 18	Placenta biopsy Any-HPV[a] negative N = 538	Unadjusted OR (95 % CI)	p-value
Placenta dysfunction syndromes[b]. N (%)					0.411[c]
No	434 (78 %)	16 (89 %)	418 (78 %)	1	
Yes	122 (22 %)	2 (11 %)	120 (22 %)	0.4 (0.05–1.90)	

Abbreviations: CI-confidence interval, HPV-human papillomavirus, *N*-total number, OR-odds ratio.

[a] Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61,90.

[b] Placental dysfunction syndromes including hypertensive disorders of pregnancy, gestational diabetes mellitus, newborns small for gestational age.

[c] Exact logistic regression model.

low in third trimester placentae (3 %), but somewhat higher in women with positive for HPV in midgestational urine (8 %). Further, high viral loads of both Any-HPV and HR-HPV in midgestational urine was associated with placental HPV. No association was seen between persistent HPV infection from midgestation to delivery and placental HPV. Only two women with placental HPV were diagnosed with placental dysfunction syndromes.

The HPV placental prevalence of 3 % is in line with several other studies that included generally healthy women that found a prevalence between 3 % and 9 % [5,15,19,22], but much lower than other studies reporting a 17 %–75 % prevalence [5,7,13,16,17]. A generally higher placental HPV prevalence, ranging from 22 % to 27 %, was reported in studies where 1) results consisted of pooled placenta results based on placenta swabs and biopsies, 2) description of how placental samples were obtained was insufficient, or 3) predominantly maternal placental tissue was tested [5,7,13,16]. This contrasts with studies where only placental biopsies were taken, with prevalence varying from 0 % to 9 % [5,15,19,22,23]. Hahn et al. reported that no women had detectable HPV in the placenta, but this lack of HPV presence may be explained by only one biopsy per placenta being tested and including only 8 placentae [23]. Comparability is a challenge as the biological samples used and methods utilized for HPV detection vary greatly. In addition to this, several of the published studies have small sample sizes, varying from 49 to 108 women with full term placental HPV results [7,13,16,23,32]. Similar to Niyibizi et al. we found that multiple midgestational HPV genotypes in urine, representing genital infections, were more common in women with placental HPV infections, 11/18 (61 %) [5]. Sarkola and colleagues found no correlation between genital HPV infection detected in the third trimester and placental HPV infection, similar to the current study where the genotype concordance between placental HPV and delivery urine HPV was lower than between placental HPV and midgestation urine genotype [19]. These observations could point to the fact that the transmission from genital HPV to placental HPV occurs in the first or second trimester of pregnancy, that is, early in the pregnancy.

In our study we found that midgestational urine HR-HPV as well as high midgestational HPV viral loads were associated with third trimester placental HPV infections. However, we did not observe an association between genotypic persistence of Any/HR-HPV infections at midgestation to delivery and placental HPV. With few previous comparable studies, we have provided valuable insight in pregnancy-related maternal HPV infection research [5,22]. Niyibizi et al. described that genital HR-HPV infection in the first trimester was associated with placental HPV in univariable regression models, in line with our study. However, Niyibizi and colleagues showed no association when adjusting for relationship status, cytology findings and genitourinary infections, in contrast to the current study [5]. To the best of our knowledge, no other investigators have published genital HPV viral loads and associations to placental infections. High viral load is known to increase the risk for persistence and potentially pathology of the cervix [33]. Lee et al. investigated 152 deliveries with placenta biopsies and concluded that persisting HPV infections during pregnancy was associated with placental HPV, in contrary to the current study [22]. Persisting HR-HPV infections during pregnancy (1st and 3rd trimester), particularly HPV16/18, was found to be associated with placental HPV infections by Niyibizi and colleagues in their adjusted analysis, this also in contrast to our findings [5]. It must be noted however that in our study, HPV16 was detected in only one placenta and HPV18 in none. In addition to this, 7/18 (39 %) placental HPV infections in our study were LR-HPV infections, which is higher than the 6.5 % reported by Niyibizi et al. [5].

In our study, only two women with placental HPV infection had a placental dysfunction syndrome, namely HDP and SGA. Slatter et al. and Gomez et al. investigated placentae with known pregnancy complications such as preeclampsia, preterm delivery and GDM, and an association to HPV infections was seen [7,17]. Gomez and colleagues showed an association between placental HPV and preterm delivery where the prevalence of placental HPV was 29/108 (27 %) and 15/29 (52 %) women with placental HPV infections delivered prematurely, whilst, placental HPV was not associated with severe preeclampsia 8/29 (28 %) [7]. Slatter et al. conducted a cross-sectional study of placentae from complicated and uncomplicated pregnancies, N = 339, and found that HPV positive cases were more frequent in women with preeclampsia and diabetes compared to HPV negative placenta (26 % versus 0.6 %) [17]. However, Gomez et al. had a retrospective study design and pregnancies with adverse pregnancy outcomes were selected in both studies, thus biased towards adverse outcomes [7,17]. Niyibizi et al. found no association between placental HPV infection and pregnancy induced hypertension and GDM [5]. It is important to note, due to the low number of women with placental HPV and adverse pregnancy outcomes in our study, firm conclusions cannot be drawn.

5.1. *Strength and limitations*

A major strength of this study is its prospective design, enabling a longitudinal follow up of the women during their pregnancy. The biological samples collected from the women were obtained and processed according to strict protocols yielding high quality samples. We obtained full thickness placental biopsies from three different areas of the placenta (peripheral, mid and central), ensuring representable biopsies and increasing the chance for HPV detection. Further, to minimize the risk for HPV contamination, placental swabs were not utilized for the detection of placental HPV as vaginal HPV could easily contaminate the surface of the placenta during the expulsion of the placenta. For the detection of genital HPV infections, we chose first-void urine samples. First-void urine samples are advantageous over mid-stream samples as the first-void also contains microorganisms from the genital tract, and not only from the bladder, as in mid-stream urine. Several investigators have shown that first-void urine samples are a feasible way of detecting genital HPV [34–36]. The extensive and detailed e-questionnaires at GW18 and 34 the enrolled women filled out provided us with valuable background information.

A limitation to this study was the lack of information regarding the women's HPV vaccination status. However, most included women were too old to be eligible for the national vaccination programs offered in Norway and Sweden (from 2009 to 2010, respectively). We do however acknowledge that some women may have been vaccinated at their own expense. Another limitation of this study was the lack of power to draw firm conclusions, as few women had placental HPV infections, as seen in the wide confidence intervals.

6. Conclusion

In 578 women from a general Scandinavian population, placental HPV was found in only 3 % at delivery, although the prevalence of midgestational genital HPV infections was nearly 40 %. However, of the 214 women with known midgestational genital HPV, 8 % proceeded to have placental HPV infections. We found midgestational urine HR-HPV and high midgestational HPV viral loads to be associated with third trimester placental HPV infections at delivery. Our study did not identify any evidence of increased risk for placental dysfunction syndromes in women with placental HPV, which should be reassuring to pregnant women. Although, few women were diagnosed with placental dysfunction syndromes and placental HPV infections, thus no firm conclusions can be drawn.

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Generative artificial intelligence technology has not been used in the preparation of this manuscript.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.05.126.

Supplementary Fig. 1: Direct Cyclical Graphs (DAG) for Aim 2: Association between HPV viral load/persistence and placental HPV.

According to Dagitty for Aim 2, covariates in the mulitivariable regression analyses were duration of preconceptional relation to child's father to estimate the total effect. Green - ancestor of exposure, Grey-other variable, White-adjusted variable, Green with black outline - exposure, Blue - outcome 5.

Mat.edu-maternal education, Mat. Age-maternal age, BMI- body mass index, Preconcept. Relation-duration of preconception relation to child's father, Alcohol consump. Pregnancy-alcohol consumption during pregnancy, smoking preg. Smoking during pregnancy.

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