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Maternal human papillomavirus infections at mid-pregnancy and delivery in a Scandinavian mother–child cohort study

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ABSTRACT

Objectives: Human papillomavirus (HPV) infections are common, especially during women's reproductive years, with unclear obstetrical impact. This study aimed to identify HPV prevalence at mid-gestation and delivery, type-specific persistence from mid-gestation to delivery, and risk factors for HPV infection and persistence.

Methods: In 757 women from a Scandinavian prospective mother–child cohort, HPV was analyzed in first-void urine samples at mid-gestation and delivery. We used Seegene Anyplex II HPV28 PCR assay for genotyping and semi-quantifying 28 genital HPV genotypes, including 12 high-risk HPVs (HR-HPV). Socio-demographic and health data were collected through e-questionnaires.

Results: Any-HPV genotype (any of 28 assessed) was detected in 38% of the study cohort at mid-gestation and 28% at delivery, and HR-HPVs in 24% and 16%, respectively. The most prevalent genotype was HPV16: 6% at mid-gestation and 4% at delivery. Persistence of Any-HPV genotype was 52%, as was HR-HPV genotype-specific persistence. A short pre-conception relationship with the child's father and alcohol intake during pregnancy increased HPV infection risk at both time points. Low viral load at mid-gestation was associated with clearance of HPV infections at delivery.

Conclusion: HPV prevalence was higher at mid-gestation compared with delivery, and low viral load was associated with clearance of HPV at delivery.

Introduction

While human papillomavirus (HPV) is a known cause of cervical cancer, its clinical impact on pregnancy, obstetrical outcomes and future non-communicable diseases is less studied. The lifetime risk for HPV infections in women is approximately 80% (McDonnold et al., 2014) and approximately 70% clear their infection within 1 year (Westrich et al., 2017). The peak prevalence of genital HPV

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infections occurs in young women, being among the most prevalent infection during reproductive years (Baseman and Koutsky, 2005; Clifford et al., 2006; Muñoz et al., 1994). As HPV targets placental trophoblast cells, HPV infections during pregnancy could potentially affect maternal and fetal outcomes negatively through abnormal placentation and placental function (Ambuhl et al., 2017).

The reported prevalence of HPV during pregnancy varies from 4% in China, 13% in Europe, to 37% in Australia (Chan et al., 2002; Domza et al., 2011; Lee et al., 2013; Liang et al., 2018; Liu et al., 2014). The Finnish Family HPV study found an HPV prevalence of 16% in the third pregnancy trimester (Louvanto et al., 2010).

Approximately 40 papillomavirus genotypes infect the human genital mucosa (Bzhalava et al., 2015; de Sanjose et al., 2018). Persistent infection with any of the 12 HPV genotypes classified as high-risk (HR-HPV) is associated with pre and malignant lesions of the cervix (Munoz et al., 2006; Walboomers et al., 1999), while other genotypes confer more unclear cancer risks (Arbyn et al., 2014; Bouvard et al., 2009; de Sanjose et al., 2010). Persistence of HPV infections is associated with both viral and host factors. Viral factors include the ability of HPV to alter local immunity in the infected cervix (Erickson et al., 2013; Song et al., 2015; Zhou et al., 2019), as well as HPV genotype and viral loads (de Sanjose et al., 2018). Host factors include ethnicity, age, cigarette smoking, number of lifetime sexual partners, age of first sexual intercourse, socio-economic status, concurrent vaginal infections, hormonal status, immunodeficiency, as well as oral contraceptive use (Erickson et al., 2013; Hariri et al., 2011; Stensen et al., 2016). Infections with HPV16 and multiple HPV genotypes seem to persist longer compared with other HPV genotypes (Louvanto et al., 2010; Rositch et al., 2013; Stensen et al., 2016). Also, HPV may establish latent infections in the basal layers of the cervix that are controlled by the host's cellular immunity. These infections can be reactivated due to alterations in the host's immune response and hormonal levels during pregnancy (Hammer et al., 2019; Leonard et al., 2016; Maglennon and Doorbar, 2012; Maglennon et al., 2014; Veress et al., 1996).

To address conflicting data on prevalence and persistence of HPV during pregnancy, we aimed to: (1) describe and compare HPV genotype-specific prevalence and persistence at mid-gestation and delivery; (2) investigate factors associated with HPV prevalence at mid-gestation and delivery as well as persistence of HPV; and (3) investigate the association of viral load at mid-gestation on HPV persistence until delivery.

Methods

Study design and subjects

The present study included all participants from the multicenter prospective PreventADALL (Preventing Atopic Dermatitis and ALLergies in children) mother–child birth cohort study (Lodrup Carlsen et al., 2018) who had available urine samples at both mid-gestation and delivery.

Briefly, pregnant women (December 2014–October 2016) from the general population were invited to participate in connection with the routine ultrasound scan at 18-week gestational age (GA) at Oslo University Hospital, Norway, Østfold Hospital Trust, Norway and Karolinska University Hospital, Sweden, as described in detail elsewhere (Lodrup Carlsen et al., 2018). Written informed consent was obtained from all women at enrollment (Lodrup Carlsen et al., 2018). Women who were not proficient in Norwegian or Swedish were excluded. Enrolled women were asked to complete comprehensive electronic (e)-questionnaires at inclusion and GA 34 weeks (Lodrup Carlsen et al., 2018). Urine samples were collected at enrollment and delivery.

Among the 2701 pregnancies (2 withdrew) recruited at mid-gestation (Lodrup Carlsen et al., 2018), 778 provided a first-void urine sample at enrollment and delivery. Among these, 757 yielded valid HPV results at both sampling time points and were included in the analysis (Supplementary Figure 1).

HPV sampling and analyses

First-void urine samples collected in 70-ml urine sample containers (Sarstedt™, Nümbrecht, Germany) at mid-gestation and delivery were used for HPV analyses. Samples were stored at 4 °C immediately after collection and, within 30 h, 4.5 ml were transferred to storage tubes (Biobanking and Cell Culture Cryogenic tubes (Nunc, Thermo Scientific™, Waltham, MA, USA) and kept frozen at −80 °C up to 36 months until analysis.

Total nucleic acids were extracted from 1000 μl thawed vortexed urine using the generic protocol on NucliSENS© easyMAG© (BioMerieux SA, Marcy l'Etoile, France), eluted in 50 μl elution buffer, and kept frozen at −80 °C until analysis.

HPV DNA was detected and genotyped using the Seegene Anyplex II HPV28 detection PCR assay (Seegene Inc. Seoul, South Korea) on the CFX96™ real-time polymerase chain reaction (PCR) system (Bio-Rad Laboratories GmbH, Munich, Germany), according to the manufacturer's instructions. The test targets 28 genital HPV genotypes in 2 separate PCR reactions, performed on 5 μl nucleic acid eluate per PCR well.

Seegene Anyplex II HPV28 provides a semi-quantitative result of HPV loads. For data analyses in this study, we categorized viral load into: *high viral load*, corresponding to positive signal for a given HPV genotype prior to cycle 40 of the total 50 cycles in the PCR (reported as either ++ or +++ in the manufacturer software); and *low viral load* corresponded to positive signal for a given HPV genotype after cycle 40 in the PCR (reported as + in the manufacturer software).

Table 1
HPV genotype classification.

Classification	HPV genotype
High-risk HPV (Carcinogen group 1), HR-HPV	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Probably/possibly high-risk HPV (Carcinogen group 2A and 2B), POSS-HR-HPV	26, 53, 66, 68, 69, 70, 73, 82
Low-risk HPV (Carcinogen group 3), LR-HPV	6, 11, 40, 42, 43, 44, 54, 61
7 High-risk HPV in the nonavalent vaccine[a], 7HR-HPV	16, 18, 31, 33, 45, 52, 58
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

Classification of the 28 HPV genotypes included in the Seegene Anyplex II HPV28 detection kit according to IARC (International Agency for Research on Cancer) (Arbyn et al., 2014; Bouvard et al., 2009).

[a] HR-HPVs included in the 9-valent vaccine, Gardasil® 9 (MSD).

Outcomes

The 28 HPV genotypes were classified in line with the International Agency for Research on Cancer (Arbyn et al., 2014; Bouvard et al., 2009) into high-risk-HPV (Group 1 carcinogen, HR-HPV), probably/possibly HR-HPV (Group 2a and 2b carcinogen, Poss-HR-HPV) and low-risk-HPV (Group 3 carcinogen, LR-HPV). We also defined a group with the 7 most carcinogenic HR-HPV genotypes (7HR-HPV) included in the nonavalent HPV vaccine, Gardasil® 9 (MSD) (HPV16, 18, 31, 33, 45, 52 and 58) (Arbyn et al., 2014) and a group with all 28 HPV genotypes detected (Any-HPV) (Table 1).

We defined persistence of HPV by at least one type-specific persistence from mid-gestation to delivery of the included HPV genotypes. We described persistence of HPV from mid-gestation to delivery for individual HPV genotypes and for the above-defined 5 groups (HR-HPV, Poss-HR-HPV, LR-HPV, 7HR-HPV, and Any-HPV).

Multiple infection was defined as being positive for more than one HPV genotype at mid-gestation and/or delivery.

Exposures

Potential risk factors were collected through e-questionnaires, including parental age and country of origin, maternal body mass index at inclusion, marital status, maternal education, living environment and family income, duration of pre-conception relationship with the child's father and, nicotine and alcohol intake during pregnancy.

Table 2
Baseline characteristics overall and stratified by HPV status at mid-gestation and delivery.

	Total N per variable[a]	Total N = 757	HPV positive at any time point N = 331 (44%)	HPV negative at both time points N = 426 (56%)
Maternal age — y. Mean (SD)	757	32.3 (4.5)	32.0 (4.6)	32.4 (4.2)
Paternal age — y. Mean (SD)	640	34.5 (5.4)	34.6 (5.4)	34.5 (5.4)
Maternal BMI — median (min–max) at inclusion	739	24.4 (17.7–48.2)	24.4 (18.4–39.3)	24.4 (17.8–48.2)
Marital status	686			
Married/cohabitants		671 (98)	287 (97)	384 (99)
Single/separated/divorced/other		15 (2)	10 (3)	5 (1)
Education (maternal)	682			
Higher education >4 y/PhD		341 (50)	136 (46)	205 (53)
Higher education ≤4 y		230 (34)	95 (32)	135 (35)
Preliminary school only (9/10 y)/high school only/other		111 (16)	63 (21)	48 (12)
Family income	675			
<600.000 NOK		118 (18)	54 (19)	64 (17)
600.000–1.000 000 NOK		332 (49)	145 (50)	187 (49)
>1.000 000NOK		225 (33)	93 (32)	132 (35)
Living environment	686			
Non-city		82 (12)	36 (12)	46 (12)
City		604 (88)	261 (88)	343 (88)
Duration of pre-conception relationship with the child's father	666			
<11 mo		22 (3)	11 (4)	11 (3)
1–2 y		69 (10)	51 (18)	18 (5)
3–5 y		160 (24)	84 (30)	76 (20)
>5 y		415 (62)	139 (48)	276 (72)
Nicotine use	686			
Nicotine never		515 (75)	607 (89)	594 (87)
Nicotine ever (any form)		171 (25)	79 (27)	92 (24)
Cigarette smoking	686			
Never		545 (79)	238 (80)	307 (79)
Before pregnancy		109 (16)	46 (16)	63 (16)
During pregnancy		32 (5)	13 (4)	19 (5)
Snus[b] use	686			
Never		539 (79)	228 (77)	311 (80)
Before pregnancy		98 (14)	41 (14)	57 (15)
During pregnancy		49 (8)	28 (9)	21 (5)
Cigarette smoking in household	686			
No		648 (95)	312 (94)	407 (96)
Yes		38 (6)	19 (6)	19 (5)
Alcohol intake	683			
Never or not during pregnancy		481 (70)	199 (67)	282 (73)
During pregnancy		202 (30)	97 (33)	105 (27)
Country of origin (mother)	686			
Norway		371 (54)	161 (54)	210 (54)
Sweden		239 (35)	104 (35)	135 (35)
Other		76 (11)	32 (11)	44 (11)
Country of origin (father)	674			
Norway		367 (55)	160 (55)	207 (54)
Sweden		248 (37)	105 (36)	143 (38)
Other		59 (9)	28 (10)	31 (8)

Abbreviations: HPV, human papillomavirus; max, maximum value; min, minimum value; mo, months; y, years; SD, standard deviation; BMI, body mass index; N, total number; NOK, Norwegian Kroner.

[a] Total number of available data in each variable.

[b] Moist snuff.

Statistical analyses

Categorical variables are presented with numbers and percentages, and continuous variables as means with standard deviations or medians with interquartile range.

We used descriptive statistics to present the prevalence of single and multiple HPV infections at mid-gestation and delivery overall and stratified by maternal age in 5-year intervals from 19 to 49 years. We applied McNemar's test to determine whether multiple infections were more common at mid-gestation compared with delivery.

We used univariable logistic regression models to investigate the association between host characteristics and HPV infection at mid-gestation and delivery. Characteristics with known associations to HPV infections from the literature were chosen. In the case of more than one significant association, we performed multivariable logistic regression models, including all significant variables in the univariable models.

We used univariable logistic regression models to investigate factors associated with type-specific persistence of HPV from mid-gestation to delivery. Only women that were type-specific HPV positive at mid-gestation were included in the analyses.

The association between viral load (dichotomous variable: high viral load ++/+++ vs low viral load +) at mid-gestation and type-specific persistence of HPV groups until delivery was examined with univariable and multivariable logistic regression models. The multivariable model included baseline characteristics significant for prevalence or persistence: cigarette smoking mother, maternal age, paternal age, living environment, paternal origin, maternal education, marital status, pre-conception relationship with the child's father, ever use of nicotine, alcohol intake mother, and cigarette smoking in household.

All models were performed based on available data (complete case analysis), and no imputation of missing values was performed. Due to missing information in single variables, the multivariable models included fewer women. We, therefore, performed univariable models in the same sample corresponding to the multivariable model to compare the significantly associated factors between the univariable and multivariable models.

All statistical analyses were performed using IBM© SPSS© statistics version 25 (Chicago, IL, U.S.A.). A *P*-value <0.05 was considered statistically significant.

Results

The mean maternal age of the 757 women included in this study was 32.3 years (SD 4.5). All pregnancies were singleton except for one twin pregnancy. Most women lived in cities (88%) and were either cohabitants or married (98%) (Table 2). Baseline characteristics were similar to women (1942) not included (but included in the PreventADALL study) (2701-2 withdrew-757 included in the current study) as shown in Supplementary Table 1.

HPV prevalence and persistence at mid-gestation and delivery

At least one of Any-HPV genotypes was detected in 291/757 (38%) of pregnancies at mid-gestation and in 209/757 (28%) at delivery. The corresponding prevalence for HR-HPV was 178/757 (24%) and 124/757 (16%). The most prevalent genotype was HPV16 at both mid-gestation, 45/757 (6%) and delivery, 33/757 (4%), followed by HPV genotypes 42, 53, 51 and 31 (Figures 1 and 2). In this study, HPV11 was not detected, while HPV6 was detected in 13/757 (1.7%) at mid-gestation and 11/757 (1.4%) at delivery, with persistence in 6/13 (46%) of cases, similar to other LR-HPVs.

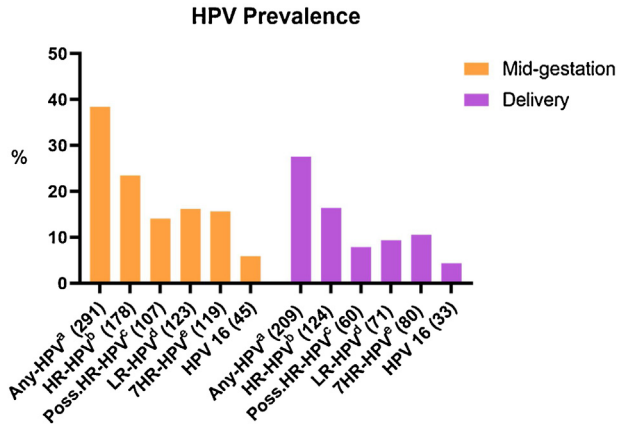

Figure 1. Distribution of HPV in different HPV genotype groups among HPV positive women at mid-gestation and delivery. The numbers in brackets represent the number of women infected with the corresponding HPV genotype.
[a]Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61.
[b]HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.
[c]Poss-HR-HPV: 26, 53, 66, 68, 69, 70, 73, 82.
[d]LR-HPV: 6, 11, 40, 42, 43, 44, 54, 61.
[e]7HR-HPV: 16, 18, 31, 33, 45, 52, 58.

The HPV prevalence stratified by age is shown in Figure 3. The highest prevalence was observed in women younger than 29 years of age, both at mid-gestation (Any-HPV: 44% and HR-HPV: 28%) and delivery (Any-HPV: 35% and HR-HPV: 21%). Women of 35–44 years showed high HPV prevalence at mid-gestation (Any-HPV: 41% and HR-HPV: 24%) and delivery (Any-HPV: 28% and HR-HPV: 17%).

Any-HPV at mid-gestation persisted in 152/291 (52%) to delivery. Type-specific persistent infection from mid-gestation to delivery was 83/178 (52%) for HR-HPV, 63/119 (53%) for 7HR-HPV genotypes, and 29/45 (64%) for HPV16 (Figure 4).

Infection with multiple HPV genotypes was observed more often at mid-gestation (43%) than at delivery (27%) ($P < 0.001$ for all HPV subgroups [Figure 2]).

Risk factors associated with HPV prevalence and persistence

Several risk factors were significantly associated with HPV prevalence at mid-gestation and delivery in univariable analyses (Supplementary Tables 2–9). Being a single-mother was significantly associated with HR-HPV infection at mid-gestation (odds ratio (OR) 2.8, 95% CI 1.0–8.0) and Any-HPV was associated at mid-gestation (OR 3.2, 95% CI 1.1–9.6) and delivery (OR 3.2, 95% CI 1.1–8.9). Low maternal level of education was associated with Any-HPV at mid-gestation and delivery, while maternal and paternal age were inversely related to 7HR-HPV infection at mid-gestation (OR 0.9, 95% CI 0.9–1.0 and OR 0.9, 95% CI 0.9–1.0 per 1 year increase in parental age). In addition, current or previous nicotine use was associated with 7HR-HPV at mid-gestation (OR 1.6, 95% CI 1.0–2.4) and with HPV16 at mid-gestation (OR 2.1, 95% CI 1.1–3.9) and delivery (OR 2.8, 95% CI 1.3–5.8). Cigarette smoking in the household was associated with 7HR-HPV at delivery (OR 2.3, 95% CI 1.0–5.2).

Two risk factors remained significantly associated with HPV prevalence in the multivariable analyses. Short duration of pre-conception relationship with the child's father was significantly associated with Any-HPV, HR-HPV and 7HR-HPV at both mid-gestation and delivery. Alcohol intake during pregnancy was associated with Any-HPV (OR 1.4, 95% CI 1.0–2.0), HR-HPV (OR 1.5, 95% CI 1.1–2.2) and 7HR-HPV (OR 1.8, 95% CI 1.2–2.7) infection at mid-gestation (Supplementary Tables 3–5, 8, 9).

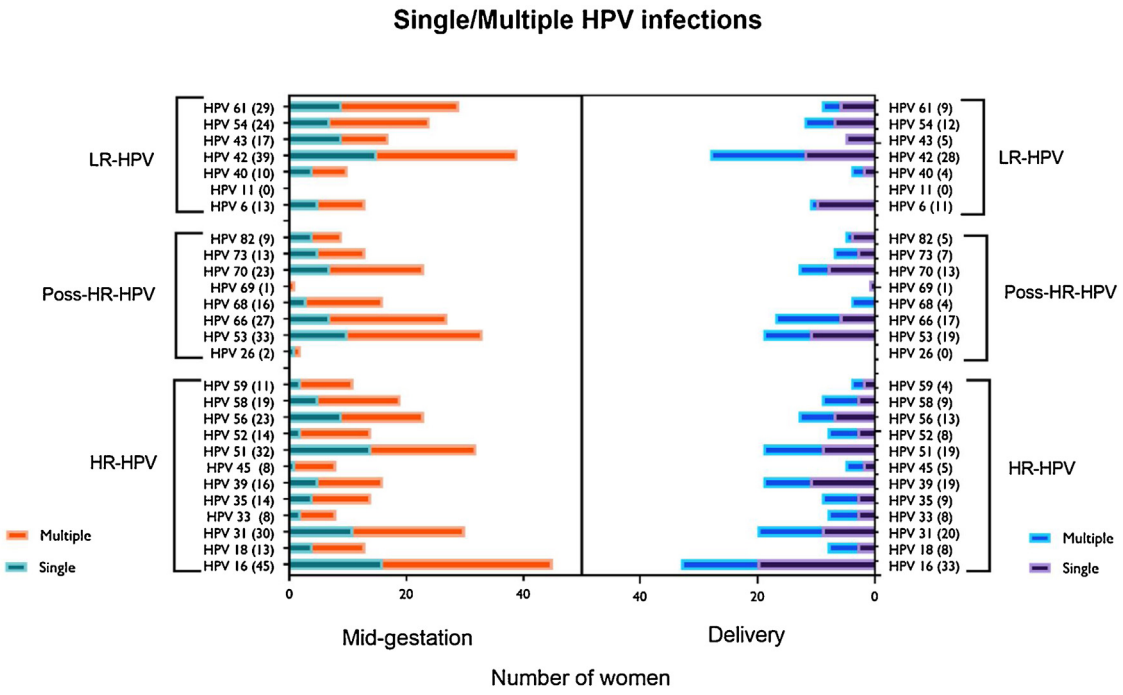

Figure 2. Number of women with single or multiple HPV infections at mid-gestation and delivery, by HPV genotype. Each bar represents the total number of women with type-specific HPV infections, divided into those with a single HPV infection and those where the type-specific HPV is a part of a multiple infection (infected with one or more type-specific HPV). The numbers in brackets represent the total number of women infected with that specific HPV (single or multiple).

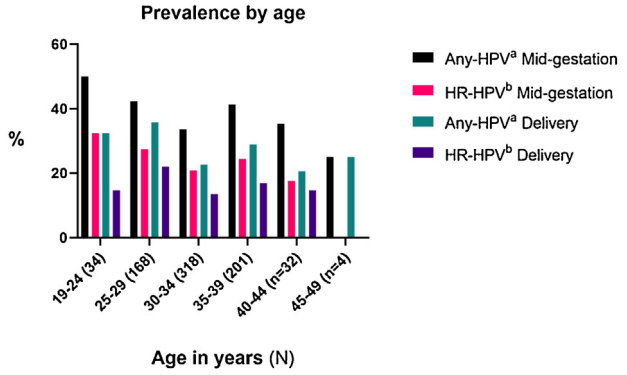

Figure 3. HPV prevalence in 757 pregnant women at mid-gestation and delivery stratified by age and HPV groups. Numbers in brackets represent the total number of women within each age group.
[a]Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61.
[b]HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.

In univariable logistic regression models (Supplementary Table 10), persistence of HPV16 was less common if the child's father originated from Sweden (OR 0.2, 95% CI 0.0–0.7). 7HR-HPV and HR-HPV persistence were higher if the mother lived in the countryside (OR 7.1, 95% CI 1.6–32.3 and OR 10.2, 95% CI 1.3–82.6, respectively), although there were only small numbers in these groups. Any-HPV persistence was inversely associated with maternal age (OR 0.95, 95% CI 0.9–1.0 per 1 year increase in age). Multivariable models were not performed, as there was only one baseline characteristic associated with persistence in each HPV group.

HPV viral load at mid-gestation and risk of persistent HPV

High viral load at mid-gestation was a predictor for persistence for all HPV groups in the univariable logistic regresssion analysis: HPV16 (OR 33.3, 95% CI 3.8–292.4), 7HR-HPV (OR 8.7, 95% CI 4.4–17.1), HR-HPV (OR 9.0, 95% CI 3.9–20.6) and Any-HPV (OR 4.5, 95% CI 2.7–7.4). The results remained statistically significant ($P < 0.001$) after adjusting for relevant confounders (Supplementary Table 11).

Discussion

In this Scandinavian prospective study of pregnancies, prevalence of Any-HPV at mid-gestation and delivery was 38% and 28%, respectively, and 24% and 16% for HR-HPV. Multiple infection was more common at mid-gestation compared with delivery. The most prevalent genotype at mid-gestation and delivery was HPV16 (6% and 4%, respectively), with persistence of 63% from mid-gestation until delivery. For Any-HPV and HR-HPV type-specific persistence was 52%. In univariable regression models, the following factors increased the risk of HPV infections (Any-HPV, HR-HPV, 7HR-HPV and/or HPV16): being a single-mother, alcohol intake during pregnancy, short duration of pre-conception relationship with the child's father, ever use of nicotine, cigarette smoking in household and low maternal educational level. In multivariable regression models, however, short duration of pre-conception relationship with the child's father and alcohol intake were the only factors that remained significantly associated with HPV infections at either mid-gestation or delivery. High viral loads at mid-gestation increased the risk of HPV persistence to delivery both in univariable and multivariable analyses.

The prevalence of Any-HPV infections in our population was higher than previously reported in a meta-analysis of Liu et al. in 13 640 women aged 30–40 years, at both mid-gestation and delivery (Liu et al., 2014). However, studies included in the meta-analysis tested for fewer HPV genotypes than in the present study. Louvanto et al. found a similar prevalence of HPV infections as in our study,

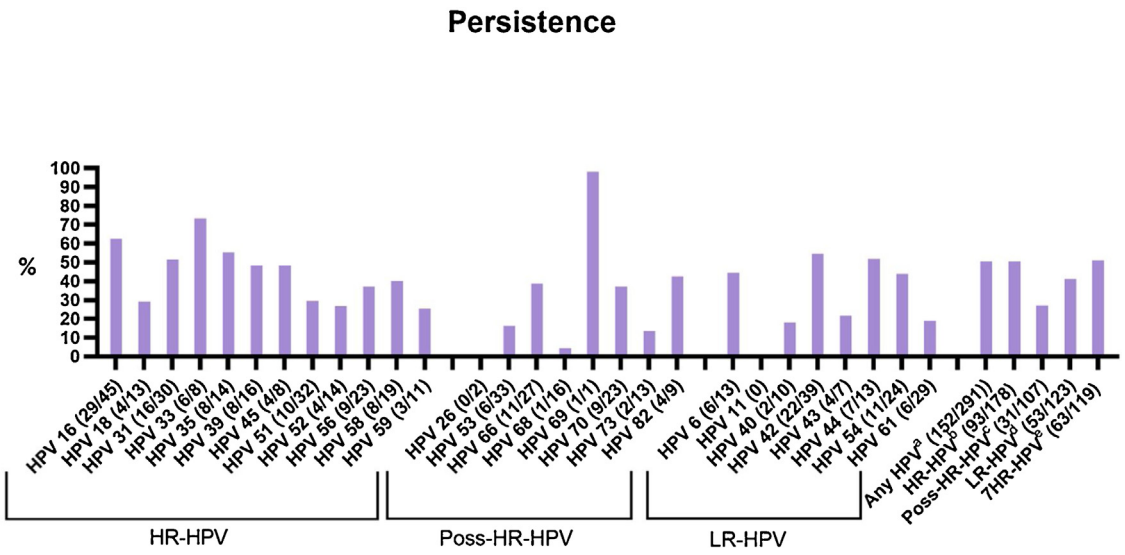

Figure 4. Proportion of persistent HPV genotypes from mid-gestation to delivery. Numbers in brackets indicate the number of HPV infections per HPV genotype that persist from the total number of HPV positive infections at mid-gestation to delivery.
[a]Any-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61.
[b]HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.
[c]Poss-HR-HPV: 26, 53, 66, 68, 69, 70, 73, 82.
[d]LR-HPV: 6, 11, 40, 42, 43, 44, 54, 61.
[e]7HR-HPV: 16, 18, 31, 33, 45, 52, 58.

stratified by ethnicity, during the third trimester (Louvanto et al., 2011), although HPV was detected from cervical samples, whereas our study used first-void urine. A Norwegian study of HPV prevalence in urine samples from young non-pregnant women showed an HR-HPV prevalence in 21-year-old women of 30% (Molden et al., 2016). This level is similar to mid-gestation prevalence in the present study population with a mean age of 32 years, indicating high HPV prevalence in older pregnant women. A meta-analysis from 2010 found a global HPV prevalence of 12% in a population of non-pregnant women with normal cytology; the most prevalent HR-HPVs globally were HPV16, 18, 52, 31, and 58 (Bruni et al., 2010). In the current study, we found HPV16, HPV51 and HPV31 as the most prevalent genotypes at both mid-gestation and delivery; HPV18 was not among the prevalent genotypes, in contrast to aforementioned studies. Our findings of HPV16 being the most prevalent genotype is in line with previous studies on HPV prevalence in both pregnant and non-pregnant women (Bruni et al., 2010; Chan et al., 2002; Liu et al., 2014; Louvanto et al., 2011, 2010; Molden et al., 2016; Smith et al., 2004; Yuill et al., 2020). The lower prevalence of HPV infection at delivery compared with mid-gestation in our study population is in line with a cross-sectional Korean study of 960 women (Kim et al., 2014). In contrast, the meta-analysis by Liu et al. reported an overall higher prevalence in the third trimester, compared with the second trimester (Liu et al., 2014), but included studies that were primarily cross-sectional and used a variety of HPV test methods. The high overall prevalence in the present study population at mid-gestation, similar to that of a 21-year-old Norwegian female population (Molden et al., 2016), could suggest reactivation of latent infections in the early stages of pregnancy. However, the significant association between short duration of pre-conception relationship with the child's father and HPV prevalence suggests that a proportion of the identified HPV infections may be newly acquired. Supporting this assumption, we found an association of being a single mother and HPV infection in the univariable analysis. HR-HPV testing for primary screening is currently being implemented in several countries for women older than 30 or 35 years, with a rationale of lower positivity rate and higher proportion of persistence vs transient infections than in younger women (Maver and Poljak, 2020). The relatively high HR-HPV prevalence during pregnancy found in our study, similar to that observed in women in their early 20s, and the fact that nearly half of the infections (except for HPV16) did not persist between the second and third trimester of pregnancy, might be important to consider for management and follow-up of pregnant women with positive HPV screening.

Previously, pregnancy was said to represent an immunodeficient state, thus increasing the prevalence of different infections (Beer and Billingham, 1971). In line with current views, Mor and Cardenas suggest that pregnancy leads to a modulated immune response, including a differentiated response to microorganisms during different stages of pregnancy where the first and third trimesters are proinflammatory, thus altering the mother's susceptibility to certain infections (Mor and Cardenas, 2010). Type-specific persistence of 52% implies an HPV clearance of nearly 50% from the second to third trimester, suggesting a proinflammatory mediated clearance. For HPV16, however, almost 2/3 of infections persisted, supporting the findings of Louvanto et al. that showed a slower rate of clearance for HPV16 (Louvanto et al., 2010). High viral loads were significantly associated with persistence, suggesting that viral factors contribute to persisting infections. It has also been suggested that the high levels of glucocorticoids during pregnancy increase the transcription and replication of HPV, further explaining the high HPV prevalence during pregnancy (Veress et al., 1996). Persistence of HPV in pregnancy has not been studied much previously. In a small study by Veress et al., 5 out of 39 women had persisting HPV infections during pregnancy (HPV test taken at early pregnancy and late pregnancy and/or after birth) (Veress et al., 1996). Smith et al. found a 56% persistence of HPV in the third trimester, although this was not type-specific persistence (Smith et al., 2004). In line with the current study, Yamasaki et al. found that multiple infections were more common in early pregnancy compared with late pregnancy (Yamasaki et al., 2011).

Our study confirmed previous findings of alcohol consumption during pregnancy being associated with the prevalence of HPV infections (Kim et al., 2014; Liang et al., 2018). Young age, high viral load, and multiple HPV infections have previously been associated

with persistence of HPV infections, in line with the present study (Bandyopadhyay and Chatterjee, 2006; Gravitt et al., 2007; Louvanto et al., 2010).

Whether prevalence and persistence of HPV infection during pregnancy, especially with HR-HPV genotypes, affects pregnancy and neonatal outcomes is unknown. A few studies have investigated the association between HPV infection and obstetrical outcomes, but the results are conflicting (Niyibizi et al., 2020). A recent study from Australia did, however, show a slight decrease in adverse pregnancy outcomes after the implementation of the HPV vaccine (Yuill et al., 2020). A recent systematic review and meta-analysis by Niyibizi et al. recommended investigation of type-specific HPV genotype and their effect on adverse pregnancy outcomes (Niyibizi et al., 2020). Our future studies will hopefully fill this knowledge gap, as we aim to assess both type-specific HPV genotype prevalence and persistence (measured in second and third trimester) as predictors for adverse pregnancy outcomes.

Strengths and limitations

A major strength of the present study is that the patient data and biological samples were from a general population-based multi-center prospective cohort. The recruitment of participants occurred in the second trimester enabling longitudinal follow-up of HPV infection until delivery, avoiding recall bias. Handling of urine samples by study team members and midwives followed strict protocols to ensure high data and sample quality. Participants provided first-void urine to maximize chances of HPV detection. Several studies have confirmed that a urine sample, in particular first-void urine, is a reliable and feasible sample type for detection of genital and cervical HPV (Jong et al., 2008; Lefeuvre et al., 2020; Pathak et al., 2014), even if HPV genotypes detected in urine samples might originate from vaginal or vulvar infections, in addition to cervical infections. We chose to use first-void urine samples to detect genital HPV infections due to sampling feasibility. The study also had several limitations; pre-conceptional and first trimester HPV status was not available, nor was information about the women's sexual history other than their present marital status. Thus, several known risk factors for HPV exposure and infection were not available (e.g., age at sexual debut or total number of sexual partners). This study lacks information on HPV status/cervical infection/abnormal cytology prior to current pregnancy; thus, the implication of possible prior disease on the observed prevalence in pregnancy is unknown. Another limitation in this study pertains to viral load measurements. Estimation of HPV loads in this study is a proxy for absolute viral load, as Seegene Anyplex II HPV28 only provides semi-quantitative data.

Conclusion

Any-HPV was observed in 38% at mid-gestation, decreasing to 28% at delivery, of pregnant Scandinavian women recruited from the general population. Short duration of pre-conception relationship with the child's father and alcohol intake during pregnancy increased the risk of HPV infections during pregnancy. Persistence of HPV varied according to genotypes from 0 to 100%, with 52% persistence for Any-HPV and 63% for HPV16. We identified high viral loads at mid-gestation as a main viral factor for persistence. The implications of HPV infections and persisting HPV infections during pregnancy are unknown and warrant further investigation in terms of pregnancy outcomes and future disease risk.

Clinical trial registration

ClincialTrials.gov number, NCT02449850.

Ethical approval

The PreventADALL study with sub-studies was approved by the Regional Ethical Committee for Medical and Health Research in South-Eastern Norway (REC; 2014/518 and REC;2017/1053) and in Sweden (2014/2242-31/4) and registered in ClinicalTrials.gov (NCT02449850). All participants in the PreventADALL study signed an informed consent form.

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Declaration of competing interests

The authors have reported no conflicts of interest.

Previous reporting of data

Parts of the reported data in this paper was presented at the EUROGIN in December 2019 congress in Monaco and at the annual 2019 (October) meeting at the Norwegian Society of Gynecology and Obstetrics in Bodø, Norway.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.05.064.

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