Vertical transmission of HPV in pregnancy. A prospective clinical study of HPV-positive pregnant women

Transmisja wertykalna HPV w ciąży. Prospektywne badanie kliniczne HPV-dodatnich ciężarnych

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Abstract

Introduction: *Human papillomavirus (HPV) is the most common sexually transmitted infection. Data reporting vertical transmission of HPV from the mother to the fetus are inconsistent and scant. Vertical transmission may occur by hematogenic route (transplacental), or by ascending contamination, or through the birth canal, which may result in the dreaded and rare laryngeal papillomatosis. Infected sperm at fertilization is a potential route of infection, too.*

Objective: *The objective of the study was to evaluate the rate of vertical transmission of HPV in HPV-positive pregnant women to their newborn infants, as well as the risk factors of HPV vertical transmission.*

Material and methods: *The clinical material was provided by 136 pregnant women, aged 18-45 years. Out of this group, 30 (22.05%) women with abnormal Pap test and positive DNA HPV test were prospectively observed. Neonatal status, i.e. DNA HPV from the nasopharyngeal smear, was recorded in all infants during the perinatal period. The conventional Pap test was performed with the cervix brush in all women. The Bethesda 2011 classification system was applied.*

Results: *An average C Reactive Protein (CRP) concentration in the studied pregnant women was 11.6083 (Std Dev – 12.93). The most frequent cytological findings in the cervical smears from the examined women were ASCUS, n=13 (43.3%), then – LSIL, n=10 (33.3%), HSIL- n=5 (16.7%) and AGC- n=2 (6.7%). In the neonates, the presence of LR HPV DNA was detected in 9 cases (30.0%) and HR HPV DNA in 7 cases (23.3%). Fourteen neonates (46.7%) tested HPV DNA negative in the perinatal period.*

Conclusions: *HPV infection (incidental or chronic) is observed in approximately 22% of pregnant women from the Małopolska province. Neonatal HPV infection in HPV-positive women was observed in 53.3% of the subjects. CRP concentration > 10 mg/dl in the serum of pregnant women statistically significantly (p 0.001) reduces the risk of vertical transmission of HPV from the mother to the fetus.*

Key words: **HPV** / **pregnancy** / **neonate** / **vertical transmission** / **CRP** /

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Streszczenie

Wstęp: *Zakażenie HPV jest najczęstszym zakażeniem przenoszonym drogą płciową (STD). Dane literaturowe dotyczące zakażenia wertykalnego HPV z matki na płód są niejednoznaczne i nieliczne. Do zakażenia wertykalnego może dochodzić drogą krwionośną (przezłożyskową) lub poprzez zakażenie wstępujące, bądź podczas przechodzenia przez kanał rodny, skutkując rzadko występującym schorzeniem- brodawczakowatością krtani. Potencjalną drogę zakażenia może stanowić również zakażone nasienie podczas zapłodnienia.*

Cel pracy: *Celem niniejszego badania była ocena częstości występowania transmisji wertykalnej HPV u HPV-dodatnich kobiet na ich nowo urodzone dzieci, jak i określenie czynników ryzyka wertykalnej transmisji HPV.*

Materiał i metody: *Materiał kliniczny stanowiło 136 ciężarnych w wieku 18-45 lat. Z tej grupy, 30 (22,05%) kobiet z nieprawidłowym wynikiem badania cytologicznego oraz dodatnim wynikiem testu na obecność DNA HPV było poddane prospektywnej obserwacji. U wszystkich noworodków zbadano wymazy z nosogardzieli w kierunku DNA HPV. U wszystkich kobiet wykonano konwencjonalną ocenę rozmazów cytologicznych w systemie Bethesda 2011 oraz oznaczono stężenie białka ostrej fazy w surowicy krwi (CRP).*

Wyniki: *Średnie stężenie CRP w surowicy ciężarnych wynosiło 11,6083 (SD- 12,93). Najczęstsze rozpoznania cytologiczne obejmowały ASCUS, n=13 (43,3%), LSIL – n=10 (33,3%), HSIL – n=5 (16,7%) i AGC- n=2 (6,7%). Obecność LR HPV DNA wykryto u 9 (30,0%) noworodków a obecność HR HPV DNA u 7 (23,3%) noworodków. U 14 (46,7%) noworodków nie stwierdzono obecności DNA HPV.*

Wnioski: *Zakażenie HPV (incydentalne i/lub przewlekłe) obserwuje się u około 22% ciężarnych w województwie małopolskim. Zakażenie HPV noworodków stwierdzane jest u 53,3% HPV- dodatnich ciężarnych. Stężenie CRP>10 mg/dl w surowicy ciężarnych w statystycznie istotny sposób (p 0,001) redukuje ryzyko zakażenia wertykalnego z HPV-dodatnich kobiet na płód.*

Słowa kluczowe: **HPV** /**ciąża** / **noworodek** / **transmisja wertykalna** / **CRP** /

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection. It is the leading causative agent of abnormal Pap smears results, and it has been confirmed to be the necessary agent of cervical carcinogenesis, leading through different stages of cervical intraepithelial neoplasia (CIN) to cervical cancer (both, squamous and adenoid type).

Up to now, more than 200 HPV genotypes have been identified, out of which approximately 40 have the affinity to affect the epithelia of the lower genital tract. A steadily growing amount of data links the majority of cancers within the oropharynx to HPV infection [1-5].

Data reporting vertical transmission of HPV from the mother to the fetus are inconsistent and scant [6-8]. Vertical transmission may occur by hematogenic route (transplacental), or by ascending contamination, or through the birth canal, which may result in the dreaded and rare laryngeal papillomatosis. Infected sperm at fertilization is a potential route of infection, too.

The extent and risk factors of HPV infections in infants remain ambiguous. Few meta-analyses to date have shown that vertical transmission developed in neonates born to infected mothers [6,7]. The rate of vertical transmission reaches up to 80% [8-10]. It is also interesting which risk factors are associated with vertical transmission in HPV-positive pregnant women.

Objectives

The objective of the study was to evaluate the rate of vertical transmission of HPV from HPV-positive pregnant women to their newborn infants, as well as to investigate the risk factors of HPV vertical transmission.

Material and methods

The clinical material was provided by 136 pregnant women, aged 18-45 years, referred to the outpatient colposcopy clinic of the University Hospital in Krakow due to abnormal Pap test results. Out of this group, 30 (22.05%) women with abnormal Pap test and positive DNA HPV test were prospectively observed. An informed consent was obtained from all subjects and the Bioethical Commission of the Jagiellonian University Medical College approved of the study.

Obstetric data, i.e. pregnancy length, mode of delivery (vaginal vs. Cesarean section), neonatal weight, and sex were obtained from all participants.

Demographic-environmental data, including place of residence (countryside vs. town), history of contraceptive pill, tobacco, and alcohol use, as well as CRP levels, were collected as well.

Neonatal status, namely – DNA HPV from the nasopharyngeal smear, was recorded in all infants during the perinatal period.

The conventional Pap test was performed with the cervix brush in all women. The Bethesda 2011 classification system was applied.

Colposcopy was performed in all women with the Olympus OCS-500 colposcope set, using terminology of IFCPC 2011 (The International Federation of Colposcopy and Cervical Pathology) identifying types of transformation zones, i.e. TZ1, TZ2, TZ3. Colposcopically directed biopsy after delivery alongside a histopathological evaluation of the cervical specimen was performed in all women.

The CRP serum level was analyzed in all pregnant women.

The material for DNA HPV analysis was sampled from the

cervix of the pregnant women and the nasopharyngeal mucosa of their neonates.

Linear Array HPV genotyping test

The LA HPV genotyping test (Roche Molecular Systems, Inc., Branchburg, NJ) is a new qualitative in vitro test for determination of 37 anogenital HPV DNA genotypes (Table (Table1).1). The LA test was applied to all samples that tested positive for HPV by DEIA and to 20 randomly selected DEIA-negative samples. (i) PCR amplification of HPV DNA.

The LA test uses biotinylated PGMY primers to amplify a 450-bp fragment within the polymorphic L1 region of the HPV genome. The PGMY amplification system has been described previously [13]. The PGMY primers are present in the "master mixture" (containing buffer, nucleotides [dATP, dCTP, dGTP, and dUTP], MgCl2, and <0.02% AmpliTaq Gold DNA polymerase) and they amplify HPV DNA from 37 HPV genotypes, including 13 high-risk types (Table (Table1).1). Amplicons incorporate dUTP, allowing the use of AmpErase enzyme (uracil N-glycosylase), which is included in the master mixture to prevent PCR carryover contamination. Capture probe sequences are located in the polymorphic regions of L1 bound by these primers. An additional primer pair targets the human β-globin gene (268-bp amplicon) to provide control for cell adequacy, extraction, and amplification.

PCR was performed in a final reaction volume of 100 µl, containing 50 µl HPV master mixture, 40 µl PCR water, and 10 µl isolated DNA. The mixture was incubated for 2 min. at 50°C and for 9 min. at 95°C, followed by 40 cycles of 30 seconds at 95°C, 1 min. at 55°C, and 1 min. at 72°C, with a final extension at 72°C lasting from 10 min. to a maximum of 1 h. The provided HPV-positive and HPV-negative controls were used with each set of 10 samples to assess the performance of the reaction [11].

Results

Study population

Mean age of the examined women was 30.97 years (Std Dev. 7.073), median 31.50. Mean number of pregnancies per woman was 2.53 (Std Dev -0.819), median 3.0. Twenty-three (76.7%) women reported smoking before conception, while 7 (23.3%) have never smoked cigarettes. Thirteen (43.3%) reported regular alcohol intake before conception, while 17 (56.7%) did not drink regularly before conception. Twenty-four (80%) women admitted to taking oral contraceptive pills for longer than three years, whereas 6 (20%) women did not take any oral contraceptive pills. Fifteen (50%) women lived in a city (Kraków) and 15 (50%) were residents of the rural areas. An average C Reactive Protein (CRP) concentration in the studied pregnant women was 11.6083 (Std Dev. 12.93). The most frequent cytological diagnoses of the cervical smears in the examined women were: ASCU: n=13 (43.3%), LSIL: n=10 (33.3%), HSIL: n=5 (16.7%), and AGC: n=2 (6.7%).

DNA of low risk HPV types- LR within the cervix was found in 17 (56.7%), co-existence of DNA of low risk- LR and high risk HPV types – HR was observed in 8 (26.6%) and the presence of DNA of high risk HPV types – HR was reported in 5 (16.7%) women. Unsatisfactory colposcopic images (TZ3) were obtained in 3 (10%) gravidas, while satisfactory colposcopic images in 27 (90%) women, among them TZ1 and TZ2 in 13 (43.3%) and in 14 (46.7%) cases, respectively. The results of histopathological examinations of the targeted specimen collected under the control of colposcopy in the post-partum period showed normal pattern of the stratified squamous epithelium and glandular epithelium of the cervix, as well as the border of the squamous and glandular epithelia in 10 (33.3%), koilocytosis in 9 (30%), CIN1 in 5 (16.6%), CIN2 in 4 (13.3%) and CIN3 in 2 cases (6.6%).

Mean birth weight of the children was 3386 grams (Std Dev. 523.039). Average length of pregnancy was 38.80 weeks (Std Dev. 1.472). In terms of sex, 14 (46.7%) females and 16 (53.3%) males were delivered. Sixteen (53.3%) and 14 (46.7%) women had vaginal deliveries and cesarean sections, respectively.

In the neonates, the presence of LR HPV DNA was detected in 9 (30.0%) and HR HPV DNA in 7 (23.3%) cases.

14 neonates (46.7%) tested negative for HPV DNA in the perinatal period.

In the univariate models, a significant correlation with vertical transmission of HPV has been shown for the CRP variable, both, when broken down into categories as well as for the continuous variable. CRP>10 mg/dl, compared to the reference category >3.1 mg/dl, statistically significantly (OR=0.007 [95% CI: 0.000-0.125]) reduces the likelihood of HPV-HR or HPV-LR

Table I. Univariate logistic regression models.

Variable		Significance	OR	95% confidence interval	
				Lower limit	Upper limit
Place of residence – town		0.148	3.000	0.676	13.309
Age		0.814	0.988	0.891	1.095
Parity		0.057	2.847	0.971	8.344
Smoking (yes)		0.148	3.889	0.617	24.517
BCP history 3 years		0.999	3230949640.076	0.000	.
CRP (continuous variable)		0.013	0.668	0.487	0.917
CRP*	CRP (< 3.1 mg/l)	0.003			
	CRP (3.1-10 mg/l)	0.075	0.077	0.005	1.296
	CRP (> 10 mg/dl)	0.001	0.007	0.000	0.125

* CRP (< 3.1 mg/dl) was adopted as the reference category.

transmission. The odds ratio of the vertical transmission of HPV for the CRP variable (continuous) was OR=0.668 [95% CI: 0.487-0.917]. Compared to the reference category >10 mg/dl, CRP<3.1 mg/dl increases the likelihood of HPV-LR transmission highly statistically significantly (OR=66 [95% CI: 3.472-1254.567]) as compared with no transmission. However, a wide confidence interval of the obtained result should be noted and taken into consideration. The odds ratio was OR = 0.711 [95% CI: 0.524-0.965].

Statistical analysis

The data were presented as means, medians, standard deviations, minimum values, maximum values and skewness.

Univariate logistic regression, multivariate logistic regression (forward selection method) and polynomial logistic regression were used in the analysis.

The level of significance $\alpha = 0.05$ was adopted in the analyses. The data were analyzed using the SPSS 20 package.

Discussion

HPV infection is the most commonly diagnosed sexually transmitted disease. Its aftermaths in the form of intraepithelial neoplasia and cervical cancer lead to high morbidity and mortality, as well as perinatal complication [12]. It is also an emotional burden for women, especially gravidas, which results in lower quality of life [13]. Although HPV infection is usually a transient, self-limiting phenomenon in pregnant women and in the general population of young women, detection of the DNA of HPV viruses of high oncogenic potential may favor the development of a persistent infection, i.e. the most important risk factor for the development of intraepithelial neoplasia and cervical cancer.

According to numerous reports of various authors, the incidence of HPV infection in the population of pregnant women amounts to 30% and is higher than in their non-pregnant peers [14, 15]. According to epidemiological studies and observations of other authors, HPV types detected most often during pregnancy include: 16, 18, 31, 33, 35 and HPV 6 and 11 [16, 17]. Pregnancy-related reduced T-cell immune response and hormonal changes affecting cellular response of the immune system, as well as the role of the GRE sequence of the HPV genome that takes advantage of changes in a woman's hormonal management during pregnancy through its analogy with the glucocorticoid receptor, may explain this epidemiological observation.

Most studies on vertical transmission of HPV infection from the mother to the fetus available in the literature comprise cohorts of pregnant women [4, 6, 7, 8, 9, 10]. The novel aim of this paper is an attempt to estimate risk factors of such transmission in women already infected with HPV and showing cytological abnormalities in their cervical smears. In our study, the percentage of neonates with detected HPV DNA was 53.3% (30.0% and 23.3% for LR and HR types, respectively). These observations are consistent with the findings of other authors [7, 14, 15, 17, 18]. Although the above-cited findings of other authors suggest that the risk of vertical transmission is relatively low in the general population of pregnant women, it increases if HPV infection in a mother is accompanied by an abnormal cytological examination result (especially HSIL and AGC), as our research has shown. Cellular lesions induced by HPV do not appear until after the infection (from 12 weeks to 2-3 years) and have a positive correlation with the so-called viral load in the cell. The logistic regression model conducted in this analysis has shown that risk factors for the vertical transmission of HPV infection from mother to fetus are: number of pregnancies greater than or equal to 3, habitual smoking, and use of oral hormonal contraception for more than three years. These observations are consistent with the epidemiological observations on risk factors for HPV infection and development of intraepithelial neoplasia of the cervix [19, 20, 21, 22, 23]. We have demonstrated in the same analysis that inflammation in the body of a pregnant woman defined by CRP level is related to the vertical transmission of HPV infection in the inversely proportional manner. Serum CRP level in pregnant women > 10 mg/dl statistically significantly (p=0.001) reduces this risk. Knowing the pathophysiology of the HPV infection as well as the idea of immune tolerance developing in pregnancy, one may speculate that inflammation in a pregnant woman triggers non-specific mechanisms which limit the proportion of HPV transmission from the mother to the fetus. This interesting observation requires further investigation and validation in larger prospective clinical trials.

Conclusion

HPV infection (incidental or chronic) is observed in approximately 22% of pregnant women from the population of the Małopolska province.

Neonatal HPV infection in HPV-positive women was noted in 53.3% of subjects.

CRP concentration >10 mg/dl in the serum of pregnant women statistically significantly (p=0.001) reduces the risk of vertical transmission of HPV from the mother to the fetus.

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