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Gintautas Domža

The influence of human papillomavirus infection on pregnant women defence systems

Summary of doctoral thesis

Biomedical science, medicine (07B)

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VILNIAUS UNIVERSITETAS

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ŽMOGAUS PAPILOMOS VIRUSO INFEKCIJOS ĮTAKA NĖŠČIOSIOS ORGANIZMO APSAUGINĖMS SISTEMOMS

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List of Abbreviations

CI - confidence interval

DNA - deoxyribonucleic acid

DTNB - 5.5'-dithiobis(2-nitrobenzoic acid)

GSH - reduced glutathione

GSSG - oxidized glutathione

GSH+GSSG - total glutathione

GSH/GSSG - ratio of both glutathione forms

GAA- general antioxidative activity

HPV - human papillomavirus

IL-10, IL-12 - interleukines

MDA- malondialdehyde

PCR - polymerase chain reaction

RR - relative risk

TBA - thiobarbituric acid

Th - T lymphocytes-helpers

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection in the world. It has been established that about 70% of the sexually active population are exposed to this infection [1]. The HPV prevalence rate among pregnant women ranges from 5.5 to 65.0 % [2]. This difference may be influenced by varied factors, including geographic factors, which means that in different countries the prevalence of the virus is not the same. Thus it is important to determine HPV prevalence data for each country.

In Lithuania, HPV prevalence studies were started in 2000. It was established, that approximately a quarter of the healthy women studied (n=1120) were infected with high-risk HPV. Most commonly, they were young and less educated women [3]. The results which were obtained during studies of women with cervical cancer in Lithuania support the fact that HPV infection is related to cervical cancer risk [4]. The morbidity from cervical cancer in Lithuania is the highest in Europe [5]. The high HPV prevalence could probably influence the high morbidity and mortality [6].

There is not much data about HPV infection in pregnant women worldwide. Furthermore, as it was mentioned before, studies on HPV infection in pregnant women have yielded inconsistent

results, including HPV prevalence, HPV clearance. Thus it is important to determine the prevalence of HPV infection in pregnant women in Lithuania, and compare it with the prevalence of this virus in other regions of the world.

On the one hand, the physiological changes during pregnancy modulate the functions of the essential protective systems (immune and antioxidative), and may increase the risk of HPV infection. These also may actuate the persistence of the virus in the cervical epithelial cells and stimulate the progression of the infection. On the other hand, HPV infection also may influence the functions of the latter systems. Thus it is important not only to determine the HPV infection rate in pregnant women, but also to evaluate the impact of this infection on the above-mentioned protective systems. The existing data supports the theory that during pregnancy, the development of oxidative stress may cause various complications of pregnancy. Increasing oxidative stress contributes to the development of the maternal hypertensive state, preeclampsia, and gestational diabetes during pregnancy [7]. Oxidative stress, however, is also important for the development of the foetus [8].

Changes in cytokines production and their functional activity are important variables when evaluating local immunity dynamics during pregnancy. We have not found in the literature data about changes of interleukins concentrations and their interface with HPV infection during pregnancy. According to the character of cytokines secretion, T lymphocytes-helpers (Th), which control the immune response, are subdivided into two subtypes (Th1 and Th2), and they suppress each others' activity. Interleukine-12 (IL-12) is one of the main factors which actuates Th1 response. Th2 lymphocytes produced interleukine-10 (IL-10) suppresses this response. In cases of viral infection, on purpose to recognise and to destroy the cells damaged by viruses, Th1 lymphocytes functions and their produced cytokines are very important. So this is why it is important to study the changes of these interleukins in pregnant women with HPV infection.

The aim of the study

To determine the impact of HPV infection on the antioxidative state of pregnant woman, and to determine the response of local immunity to this infection.

The study goals

In order to achieve this aim the following goals were:

1. to determine HPV infection in pregnant women, to determine its types and to compare infection prevalence between first and third trimesters of pregnancy;
2. to study the antioxidative system state during pregnancy due to HPV infection;

3. to study interleukins (IL-10 and IL-12), which show local immunity, concentration changes during pregnancy due to HPV infection.

Scientific novelty

Considering the high morbidity and mortality from cervical cancer in Lithuania, our established HPV infection in pregnant women and identified types of this virus enabled us to determine changes of HPV infection during pregnancy in the high risk cervical cancer population. Also there is possibility to compare obtained data with that from authors in other countries.

It is established that women develop oxidative stress during pregnancy, and that its level does not depend on HPV infection.

It is established that changes in IL-10 and IL-12 concentrations in cervicovaginal washing fluid (local immunity) are related to Th1 and Th2 lymphocytes function, and depend on HPV infection.

Materials and Methods

The study objects. 213 pregnant women who were attending one of the centres of the Central Outpatient Clinic of Vilnius city (Lithuania) in 2008-2010 were recruited. All women attended the clinic for routine gestational control. The changes in participant numbers during the study are shown on Figure 1.

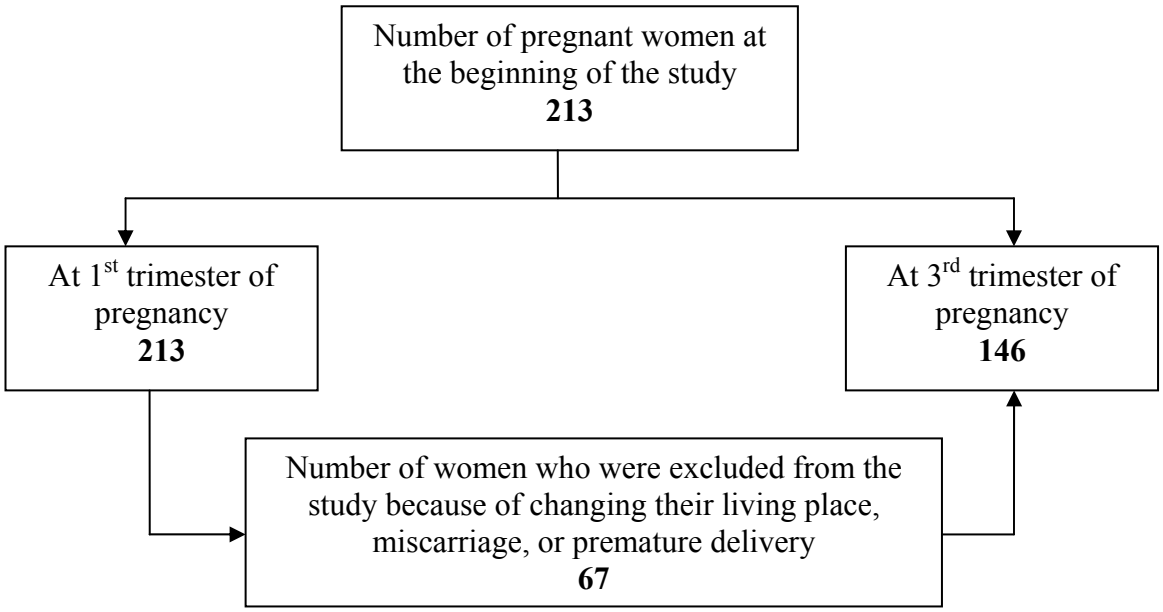


Figure 1. The changes in participant numbers during the study.

These women were examined for HPV infection and its type. Tests were performed in the first and third trimesters of pregnancy. The participating women had to answer a questionnaire with 22 questions. There were questions about social demographic features (age, education, marital status, nationality, social status), sexual behaviour (first sexual experience, the number of sexual partners, the extra-marital sexual contacts of the partner), gynaecological history (number of deliveries and abortions, first menses, gynaecological diseases in the past, use of contraception, results of cytological cervical smears). To perform the study, the permission of the Lithuanian Bioethics Committee was obtained (2008-03-26, No 21).

HPV identification and its typing. HPV infection was detected in the cervicovaginal washing fluid. During the gynaecological examination the cervix was washed with 10 ml of saline. The material was collected in a sterile container that was placed in ice and transported to the laboratory for analysis. In the laboratory, DNA was extracted from the material and tests for HPV were performed. The material was stored at -20ºC and was prepared for the test in a few stages. First, sample DNA was extracted using the commercial DNA extraction kit

Sorpoclean (Joint-stock company SORPO Diagnostics, Lithuania) following the recommendations of the manufacturer.

Considering the published data [9], HPV types were grouped in several categories: group I included type 16 HPV that determines about 54.6 % of cervical cancer cases; group II included type 18 HPV (18.8 % cases); group III –types 31, 52, and 58 HPV (1.1-4.4 % cases); group IV –types 33, 39, 45, 51, and 56 HPV (0.2-0.8 % cases); group V – several HPV types; group VI – not identified HPV types.

Polymerase chain reaction (PCR). PCR was performed to identify HPV using 50 µl of solution containing 45 µl of commercial *HPV Master Mix* solution (Joint-stock company SORPO Diagnostics, Lithuania) and 5 µl of DNA sample in accordance with the recommendations of manufacturer. DNA was examined to ensure the presence of ß globin gene in all samples prior to PCR for HPV identification as described [10]. Each PCR analysis to identify HPV was performed using positive control from the commercial kit. For negative control samples without DNA (with de-ionized water) were used. HPV positive samples were further examined: additional PCR were performed to identify the type of virus using commercial *HPV 16, 18 Master Mix, HPV 31, 33, 39, 51, 52, 56, 58, 59 Master Mix,* and *HPV 6/11, 45 Master Mix* kits (Joint-stock company SORPO Diagnostics, Lithuania).

Visualization PCR products by electrophoresis. The amplified PCR products were analyzed by electrophoresis. Electrophoresis was performed on 2 % agarosis gel, the gel was visualized by ethidium bromide and products were analyzed in a UV transilluminator (320 nm). Pictures of the results were taken and stored in hardware.

Analysis of antioxidative system parameters. Antioxidative system parameters were determined in two samples of blood plasma and cervicovaginal washing fluid collected at the first and the third trimesters of pregnancy, respectively. The level of lipid peroxidation product malondialdehyde (MDA; nmol/ml) was determined by thiobarbituric acid (TBA) assay based on the release of color MDA/TBA complexes as described [11]. The concentration of functional antioxidant glutathione (mkmol/ml) both in reduced (GSH) and oxidized (GSSG) form was detected using a recycling system by 5.5'-dithiobis(2-nitrobenzoic acid) (DTNB) [12]. Analytes were pre-treated with 2-vinylpiridine to determine the concentration of GSSG. The GSH/GSSG ratio was calculated. general antioxidative activity (GAA; %) was determined in the test of Tween 80 oxidation. The reaction mixture containing 0.2 ml of blood plasma, 2 ml of 1 % Tween 80 aqueous solution, 0.2 ml of 1 mM ferrum sulphate solution and 0.2 ml of 10 mM ascorbic acid solution was incubated for 48 hours in temperature at a 40ºC. The cool reaction mixture then was added to 1 ml of 40 % tricholacetic acid and was stored for one hour

at room temperature. The mixture was centrifuged for 15 min at 8000 rpm, supernatant was removed to 2 ml of 0.25 % TBA, and the mixture was heated for 15 min. The cool mixture then was analysed for absorption at 532 nm for MDA content. All parameters were analyzed spectrophotometrically.

Analysis of local imune system parameters. Local immune system parameters were determined in cervicovaginal washing fluid collected in the first and the third trimesters of pregnancy.

The cervicovaginal washing fluid was assayed for cytokines interleukin IL-10 and IL-12 by enzyme-linked immunosorbent assay (ELISA) using commercial *BioSourse Human IL-10-Easia* and a *BioSourse Human IL-12+p40 Easia ELISA* kit following the recommendations of the manufacturer.

Statistical analysis. A statistical analysis was performed using the statistical software package SPSS (v.17) and Microsoft Office Excel 2007. All variables under analysis were qualitative. As almost two thirds of the data did not pass the normality test, the differences between mean values of the variables were tested using non-parametric tests: the Mann–Whitney U test (presented with Z statistics values) and the Wilcoxon sign rank test for the independent samples and for the dependent samples, respectively. The level of significance was set at 0.05.

Results

General Characteristics

213 pregnant women who agreed to participate in the study and signed an Informed Consent Form were involved in the study. 76.5 % (n=163) of all the women involved in the study were married. 58.7 % (n=125) were officials, 77.5 % (n=165) had a higher education. The majority of the women were Lithuanians, at 81.2 % (n=173). 30.5 % (n=65) of the pregnant women had had their first sexual relationship when they were less than 18 years old. About half of the participants (55.4 %, n=116) had had from one or two partners. According to contraceptive method, 52.6 % (n=112) of the women used hormonal contraceptives, and 39.9 % (n=85) used other methods. 69.5 % (n=148) of the women had gynaecological diseases anamnesis. Because of cervical intraepithelial changes, four women had had cervical conization before their pregnancy.

HPV Infection and HPV Types

At the beginning of the study (1st trimester of pregnancy), 213 pregnant women were HPV infected 17.8% (n=38) (95% CI = 13.25÷23.57). Among identified HPV types, 7.8% of

the pregnant women had HPV types 16 and 18, and 13.2 % had several HPV types (HPV 16 – 2 cases; HPV 31 – 1 case); 21.1 % of the pregnant women had HPV types 31, 52 and 58 (HPV 31 – 3 cases; HPV 52 – 3 cases; HPV 58 – 2 cases), which, according to the literature [9], individually accounts for 1.1 % to 4.4 % of cases of cervical cancer; 10.5 % of the pregnant women had HPV types 33, 39, 51 and 56 (HPV 33 – 1case; HPV 39 – 1 case; HPV 51 – 1 case and HPV 56 – 1 case), which individually accounts for 0.2 % to 0.8 % of cases of cervical cancer; the remaining 47.4 % were not identified HPV types (Table 1).

Table 1. Types of HPV in first trimester of pregnancy

Group	HPV type		n	%
I	16		2	5.2
II	18		1	2.6
III	31, 52, 58	31	3	21.1
		52	3	
		58	2	
IV	33, 39, 51, 56	33	1	10.5
		39	1	
		51	1	
		56	1	
V	Several HPV types:		5	13.2
	16,31,56,58		1	
	16 and 45		1	
	31 and 51		1	
	52 and 36		1	
	52 and 58		1	
VI	Unidentified HPV types		18	47.4
Total			38	100.0

67 women during the third trimester of pregnancy did not show up to a repeat examination of cervicovaginal washing fluid for HPV: they had changed their place of residence, had had a miscarriage, or a preterm delivery. It should be noted that 14 of them had HPV infection in the first trimester of pregnancy. Therefore, 146 pregnant women were studied in their third trimester of pregnancy, among them 10.3 % (n=15) (95 % CI=6.76÷17.16) were identified with HPV. The distribution of different HPV types is shown in Table 2.

Table 2. Types of HPV in third trimester of pregnancy

Group	HPV type	n	%
I	16	2	13.3
II	18	2	13.3
III	58	3	20.0
IV	33, 39, 51, 56	0	0
V	Several HPV types:	3	20.0
	16 and 45	1	
	16, 18 and 33	1	
	31 and 51	1	
VI	Unidentified HPV types	5	33.7
Total		15	100.0

Changes in HPV infection regarding the trimester of pregnancy. With the aim of comparing the changes in HPV infection regarding the trimester of pregnancy, the data incorporated only women with whom HPV was investigated in both the first and third trimesters of the pregnancy. There were 146 such women, 17.8 % (n=26) of them had HPV (95 % CI=12.4÷24.87). It should be noted that 61.5 % (n=16) of these were not determined as infected with HPV in the third trimester and five women were newly infected during the pregnancy (Fig. 2).

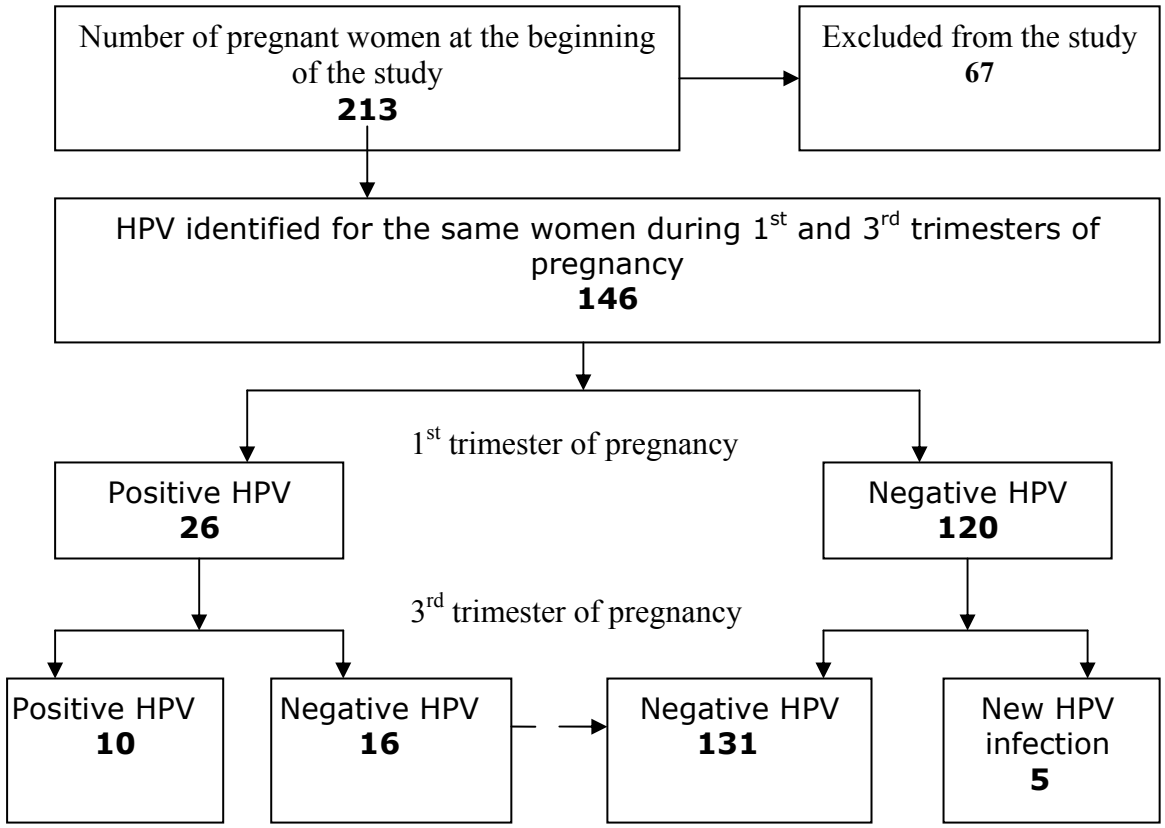


*Figure 2***.** Changes in HPV infection during pregnancy

For 10/26 women, the HPV infection persisted; 16/26 women were stated as clear of infection.

Contamination with HPV according to the different virus types is shown in Table 3.

Table 3. Changes of HPV types during pregnancy

Group	HPV type	1st trimester of pregnancy (n)	3rd trimester of pregnancy (n)	
			persistency of infection	new infection
I	16	2	1	1
II	18	1	1	1
III	31, 52, 58	6	2 (58 type)	1 (58 type)
IV	39, 51, 56	3	0	0
V	Several types:	3	2	1 (16, 18 and 33 types)
	16 and 45	1		
	31 and 51	1		
	52 and 36	1		
Total		15	6	4

n - number of pregnant women.

At the beginning of the study (first trimester of pregnancy), 11 women were infected with an unidentified HPV type. In the third trimester of pregnancy, seven of them were stated as clear of infection and one woman was newly infected. In the third trimester of pregnancy, the persistent HPV infection were types 16, 18, 58 and several other (16 and 18; 31 and 51) types. For the women who were infected during pregnancy, types 16, 18 and 58, and mixed infection (16, 18 and 33 HPV types) were identified. The women who were determined as being high risk HPV types would also be followed up after delivery, because they were at a higher risk of cervical cancer.

The relative risk of HPV infection for pregnant women. According to the data shown in Table 4 (analyses are based on data from women with HPV infection in the first trimester only), women younger than 29 years of age (RR=0.291; 95 % CI=0.186÷0.456; p=0.063), and women who had gynaecological diseases in amamnesis (RR=0.383; 95 % CI=0.222÷0.659; p=0.013), have a higher risk of testing positive for HPV.

Table 4. The risk of HPV infection for pregnant women

Feature		Positive HPV test 38 tarp 213	Contamination with HPV (%)	RR	95% CI
Age	<29	25/111	22.5	1	reference
	>29	13/102	12.7	1.77	0.858 – 3.6338
	χ^2 p- value	0.06			
Marital status	Not married	6/42	14.3	1	reference
	Married	28/163	17.2	0.832	0.323 – 2.139
	Divorced	2/3	66.7	0.214	0.029 – 1.557
	Cohabiting	2/5	40	0.357	0.056 – 2.27
	χ^2 p- value	0.07			
Age of first sexual relation	<18	13/65	20.0	1	reference
	18-20	16/91	17.6	1.138	0.512 – 2.527
	>20	9/57	15.8	1.267	0.504 – 3.183
	χ^2 p- value	0.83			
Number of sex partners over life	1	5/58	8.6	1	reference
	2	11/60	18.3	0.47	0.154 – 1.437
	3-5	16/72	22.2	0.388	0.134 – 1.12
	>5	6/23	26.1	0.333	0.092 – 1.19
	χ^2 p -value	0.15			
Sexually transmitted diseases in anamnesis	Yes	7/32	21.9	1	reference
	No	31/181	17.1	1.277	0.518 – 3.148
	χ^2 p- value	0.52			
History of gynaecological disease	Yes	20/148	13.5	1	reference
	No	18/65	27.7	0.488	0.242 – 0.983
	χ^2 p- value	0.01			
Smoking	Yes	6/34	17.6	1	reference
	No	32/179	17.9	0.987	0.383 – 2.542
	χ^2 p- value	0.97			
Use of hormonal contraception	Yes	21/112	18.7	1	reference
	No	17/101	16.8	1.114	0.557 – 2.229
	χ^2 p -value	0.72			
Use of other contraceptive methods	Yes	12/85	14.1	1	reference
	No	26/128	20.3	0.695	0.333 – 1.452
	χ^2 p- value	0.25			

RR - relative risk, CI - confidence interval.

The Status of the Antioxidative System in Pregnant Women due to Infection with HPV

The analysis started by comparing antioxidative system variables for all women (both HPV-infected and not-infected) in the first and the third trimesters of the pregnancy. The results are presented in Table 5.

It is evident that a significant difference was determined for most variables of the antioxidative system (amount of reduced (GSH), oxidized (GSSG) and total glutathione (GSH+GSSG), amount of malondialdehyde (MDA) and general antioxidative activity (GAA)) in the blood plasma of pregnant woman, if comparing trimesters of the pregnancy. The concentration of all glutathione forms and GAA were reduced during the pregnancy, while the MDA level was enhanced. On the other hand, only the average level for two of those variables (amount of reduced and total glutathione) changed significantly during the development of the pregnancy if the cervicovaginal washing fluid is analyzed.

Table 5. Antioxidative system variables in blood plasma and cervicovaginal washing fluid of all pregnant women involved in the study. Variables were compared in the first (I) and the third (III) trimesters

Parameter	Trimester	Mean	N	Standard deviation	Wilcoxon test	Significance
Blood plasma						
GSH	I	0.50	60	0.21	-5.30	0.00
	III	0.28	60	0.12		
GSSG	I	0.29	60	0.13	-6.24	0.00
	III	0.13	60	0.05		
GSH+GSSG	I	0.79	60	0.28	-6.00	0.00
	III	0.42	60	0.14		
GSH/GSSG	I	2.33	60	2.60	-1.82	0.07
	III	2.34	60	1.29		
MDA	I	7.12	62	3.05	-5.97	0.00
	III	12.62	62	4.34		
GAA	I	30.96	62	10.78	-2.51	0.01
	III	27.45	62	9.12		
Cervicovaginal washing fluid						
GSH	I	0.60	65	0.53	-3.94	0.00
	III	0.32	65	0.12		
GSSG	I	0.27	65	0.15	-1.55	0.12
	III	0.21	65	0.10		
GSH+GSSG	I	0.87	65	0.62	-3.91	0.00
	III	0.53	65	0.17		
GSH/GSSG	I	2.38	65	1.44	-1.72	0.09
	III	2.00	65	1.33		
MDA	I	4.31	65	4.02	-1.76	0.08
	III	3.54	65	5.27		
GAA	I	23.38	64	9.60	-1.63	0.10
	III	26.28	64	10.54		

GSH - reduced glutathione (mkmol/ml), GSSG - oxidized glutathione (mkmol/ml), GSH+GSSG - total glutathione (mkmol/ml), GSH/GSSG - ratio of both glutathione forms, MDA - malondialdehyde (nmol/ml), GAA - general antioxidative activity (%).

Here it should be suggested that the organism level (blood plasma) showed the status of the antioxidative system of pregnant women better than the local level (cervicovaginal washing fluid), even taking into account the fact that different values of each parameter were detected comparing both fluids. The reason might be that the cervix is possibly more protected from changes of essential functions than the whole organism during pregnancy. A significant drop in the average GSH level and a increase in the average MDA level in the blood plasma during a developing pregnancy confirmed the presence of oxidative stress.

In the second stage of the analysis pregnant women were grouped considering HPV infection (first group HPV-positive, and second group HPV-negative). Antioxidative system variables were analyzed within each group comparing data between two trimesters of the pregnancy. The results of the HPV-positive group and the HPV-negative group are summarized in Table 6 and Table 7 respectively.

Table 6. Antioxidative system variables in blood plasma and cervicovaginal washing fluid of HPV-positive pregnant women involved in the study. Variables were compared in the first (I) and the third (III) trimesters

Parameter	Trimester	Mean	N	Standard deviation	Wilcoxon test	Significance
Blood plasma						
GSH	I	0.52	20	0.19	-3.06	0.00
	III	0.31	20	0.14		
GSSG	I	0.22	20	0.10	-2.84	0.00
	III	0.13	20	0.04		
GSH+GSSG	I	0.74	20	0.23	-3.19	0.00
	III	0.43	20	0.15		
GSH/GSSG	I	3.47	20	3.92	-0.37	0.71
	III	2.53	20	1.49		
MDA	I	7.97	22	3.89	-3.26	0.00
	III	12.65	22	4.35		
GAA	I	31.15	22	12.91	-1.87	0.06
	III	26.70	22	8.81		
Cervicovaginal washing fluid						
GSH	I	0.61	26	0.43	-2.81	0.00
	III	0.31	26	0.12		
GSSG	I	0.25	26	0.14	-1.28	0.20
	III	0.20	26	0.10		
GSH+GSSG	I	0.86	26	0.51	-2.91	0.00
	III	0.51	26	0.15		
GSH/GSSG	I	2.65	26	1.60	-1.63	0.10
	III	2.07	26	1.62		
MDA	I	4.92	26	4.44	-0.92	0.36
	III	4.60	26	7.69		
GAA	I	22.11	25	9.60	-0.44	0.66
	III	23.85	25	10.54		

GSH - reduced glutathione (mkmol/ml), GSSG - oxidized glutathione (mkmol/ml), GSH+GSSG - total glutathione (mkmol/ml), GSH/GSSG - ratio of both glutathione forms, MDA - malondialdehyde (nmol/ml), GAA - general antioxidative activity (%).

Table 7. Antioxidative system variables in blood plasma and cervicovaginal washing fluid of HPV-negative pregnant women involved in the study. Variables were compared in the first (I) and the third (III) trimesters

Parameter	Trimester	Mean	N	Standard deviation	Wilcoxon test	Significance
Blood plasma						
GSH	I	0.49	40	0.23	-4.29	0.00
	III	0.27	40	0.11		
GSSG	I	0.32	40	0.13	-5.48	0.00
	III	0.13	40	0.05		
GSH+GSSG	I	0.81	40	0.31	-5.09	0.00
	III	0.41	40	0.14		
GSH/GSSG	I	1.76	40	1.33	-2.61	0.01
	III	2.25	40	1.18		
MDA	I	6.65	40	2.39	-4.97	0.00
	III	12.61	40	4.39		
GAA	I	30.86	40	9.59	-1.84	0.07
	III	27.86	40	9.37		
Cervicovaginal washing fluid						
GSH	I	0.60	39	0.59	-2.86	0.00
	III	0.33	39	0.12		
GSSG	I	0.28	39	0.16	-1.15	0.25
	III	0.22	39	0.10		
GSH+GSSG	I	0.87	39	0.70	-2.65	0.01
	III	0.55	39	0.18		
GSH/GSSG	I	2.20	39	1.31	-0.77	0.44
	III	1.96	39	1.12		
MDA	I	3.90	39	3.71	-1.48	0.14
	III	2.84	39	2.56		
GAA	I	24.19	39	9.60	-1.74	0.08
	III	27.84	39	10.54		

GSH - reduced glutathione (mkmol/ml), GSSG - oxidized glutathione (mkmol/ml), GSH+GSSG - total glutathione (mkmol/ml), GSH/GSSG - ratio of both glutathione forms, MDA - malondialdehyde (nmol/ml), GAA - general antioxidative activity (%).

It is worth noting that GAA in blood plasma was not significantly changed if comparing two trimesters in each of these two groups, although the difference in that parameter was significant in the group of all women tested. Moreover, the GSH/GSSG ratio significantly increased for HPV-negative women due to a nearly three times lower GSSG concentration. Despite the fact that the variable was also lower in the third trimester of HPV-positive women, the difference was not so noticeable. Consequently, the GSH/GSSG ratio decreased in that group.

The same variables (GSH and GSH+GSSG level) were significantly decreased in the cervicovaginal washing fluid, if comparing the two trimesters of pregnancy in all groups.

In the third stage of the study, HPV-positive women (N=26) were compared with HPV-negative women (N=40) of a similar average age (30 years). The results are summarized in Table 8.

Table 8. Antioxidative system variables in blood plasma and cervicovaginal washing fluid comparing HPV-negative and HPV-positive pregnant women. Variables were compared between two groups in the first and the third trimesters separately

Parameter	Mean	Standard deviation	Mean	Standard deviation	Mann-Whitney U	Z statistics	Significance
	HPV-negative		HPV-positive				
Blood plasma							
Age	30.40	4.24	29.34	7.41	622	-0.42	0.67
GSH1	0.49	0.23	0.55	0.20	561.5	-1.47	0.14
GSH2	0.27	0.11	0.29	0.14	476.5	-0.05	0.96
GSSG1	0.32	0.13	0.23	0.11	466	-2.49	0.01
GSSG2	0.13	0.05	0.14	0.08	474.5	-0.08	0.94
GSH+GSSG1	0.81	0.31	0.78	0.24	680	-0.21	0.83
GSH+GSSG2	0.41	0.14	0.48	0.33	450.5	-0.41	0.68
GSH1/GSSG1	1.76	1.33	3.96	4.78	392	-3.27	0.00
GSH2/GSSG2	2.25	1.18	3.01	3.14	394.5	-1.19	0.24
MDA1	6.65	2.39	8.01	3.55	627.5	-1.33	0.19
MDA2	12.61	4.39	13.40	5.32	458	-0.31	0.76
GAA1	30.86	9.59	31.49	11.67	733	-0.27	0.79
GAA2	27.86	9.37	26.25	8.87	418	-0.60	0.55
Cervicovaginal washing fluid							
Age	30.40	4.24	29.34	7.43	622	-0.42	0.67
GSH1	0.59	0.59	0.67	0.58	744.5	-0.35	0.73
GSH2	0.33	0.12	0.30	0.13	443.5	-1.08	0.28
GSSG1	0.27	0.16	0.26	0.15	744	-0.35	0.72
GSSG2	0.22	0.10	0.22	0.11	517	-0.12	0.90
GSH+GSSG1	0.86	0.69	0.93	0.69	761	-0.19	0.85
GSH+GSSG2	0.55	0.18	0.66	0.58	520.5	-0.08	0.94
GSH1/GSSG1	2.17	1.31	2.58	1.57	646.5	-1.31	0.19
GSH2/GSSG2	1.96	1.12	2.07	1.60	489.5	-0.48	0.63
MDA1	3.83	3.69	4.40	4.15	754.5	-0.44	0.66
MDA2	2.84	2.56	5.60	8.86	436	-0.95	0.34
GAA1	24.08	9.43	23.83	10.19	792	-0.08	0.94
GAA2	27.84	10.44	23.96	10.64	380	-1.25	0.21

GSH - reduced glutathione (mkmol/ml), GSSG - oxidized glutathione (mkmol/ml), GSH+GSSG - total glutathione (mkmol/ml), GSH/GSSG - ratio of both glutathione forms, MDA - malondialdehyde (nmol/ml), GAA - general antioxidative activity. 1 - the first trimester, 2 - the third trimester.

In the first trimester the GSSG level in blood plasma was significantly lower for HPV-infected women. No significant differences in any variable of the antioxidative system were determined in the cervicovaginal washing fluid when comparing each trimester separately. Variables in blood plasma also showed no significant differences in the third trimester.

An enhanced level of MDA in the third trimester of pregnancy for all women as well as for HPV-infected and not-infected women separately was determined. Only slightly higher values of the variable were determined due to HPV infection. The result could mean that lipid peroxidation was one of the most sensitive processes affected by systemic oxidative stress during pregnancy, although it did not markedly depend on the fact of infection.

For confirmation that pregnancy plays an essential role for the level of MDA, the blood plasma of pregnant women (N=115, average age 31 years) was compared with the plasma of not pregnant women (N=58, average age 35 years). The results are summarized in Table 9.

Table 9. Malondialdehyde level in blood plasma of pregnant and not pregnant women. Parameters were compared between two groups in first and third trimester of the pregnancy separately

Parameter	Mean	Standard deviation	Mean	Standard deviation	Mann-Whitney U	Z statistics	Significance
	Pregnant women (N=115)		Not pregnant women (N=58)				
Age	31	3.83	35	7.89	2301.5	-3.12	0.00
MDA1	8.64	3.79	7.18	2.85	2628.5	-2.27	0.02
MDA2	13.19	3.87			809	-8.12	0.00

MDA – malondialdehyde (nmol/ml). 1- I trimester, 2 – III trimester.

In both the first trimester and in the third trimester of pregnancy it is evident that the MDA level is significantly different comparing with the not pregnant state. It was found that in the first trimester this parameter for pregnant women was close to the parameter for not pregnant women, while during the developing pregnancy the MDA level increased 1.6 times (Table 9). With this, we confirm that pregnancy influences the degree of lipid peroxidation. As the MDA level is recommended to be tested in the course of the development of the fetus particularly in the case of restricted fetus growth, it could also be suggested to be tested during a normal pregnancy.

The Status of the Local Immune Response in Pregnant Women due to Infection with HPV

IL-10 and IL-12 changes during pregnancy were investigated in cervicovaginal washing fluid. In order to evaluate the impact of HPV infection on changes of the above-mentioned interleukins, IL-10 and IL-12 concentrations between HPV-infected and non-infected pregnant women in the first trimester of pregnancy were compared (Table 11).

Table 11. IL-10 and IL-12 concentrations HPV infected and non-infected pregnant women in the first trimester

Trimester of pregnancy	Parameter	HPV-infected in 1st trimester			HPV non-infected in 1st trimester			Wilcoxon test	Significance
		N	Mean	Standard deviation	N	Mean	Standard deviation		
1st	IL-10 (pg/ml)	15	1.97	1.64	28	0.01	0.00	437	0.00
	IL-12 (pg/ml)	14	1.09	1.48	20	2.47	2.21	177	0.02
3rd	IL-10 (pg/ml)	15	1.45	1.45	28	0.00	0.00	449	0.00
	IL-12 (pg/ml)	14	0.80	1.66	20	1.93	2.13	184	0.03

Significant differences in concentrations of both interleukins were stated between the investigated groups. The IL-10 concentrations were significantly higher in the HPV-infected pregnant women group, compared with the concentration of this interleukin in the non-infected pregnant women group. The IL-12 concentration was lower during both trimesters of pregnancy. The data obtained show that if there is no HPV infection in the third trimester of pregnancy, the functions of local cellular immunity (Th1) were not activated.

Thereinafter, with the purpose of confirming that HPV infection has an impact on the local immune system response to this infection, investigated interleukins concentrations were compared in both the first and third trimesters of pregnancy between HPV-infected and non-infected pregnant women (Table 12).

Table 12. IL-10 and IL-12 concentrations in HPV-infected and non-infected pregnant women on both trimesters

Trimester of pregnancy	Parameter	HPV-infected			HPV non-infected			Wilcoxon test	Significance
		N	Mean	Standard deviation	N	Mean	Standard deviation		
1st	IL-10 (pg/ml)	10	1.95	1.64	28	0.01	0.00	450	0.00
	IL-12 (pg/ml)	9	1.61	1.76	20	2.47	2.21	111	0.26
3rd	IL-10 (pg/ml)	10	1.82	1.43	28	0.00	0.00	443	0.00
	IL-12 (pg/ml)	9	2.04	2.67	20	1.93	2.13	134	0.96

It was found that the IL-10 concentration in the cervicovaginal washing fluid of infected women during the first trimester of pregnancy was significantly higher as against the concentration of this interleukin in the cervicovaginal washing fluid of HPV non-infected women, and during the third trimester of pregnancy IL-10 in non-infected pregnant women group was not detected at all. Thus the IL-10 concentration in cervicovaginal washing fluid differences during both the first and the third trimesters of pregnancy between HPV infected and non-infected women are significant. It shows that Th2 lymphocytes (suppress cellular immunity response) function was activated in infected women.

Comparing women groups in whom HPV infection in the third trimester of pregnancy was not determined and in whom HPV persisted, it was established, that in the latter group Th1 response was attenuated (IL-12 level increased), and anti-inflammatory IL-10 production in fact was not alternated; in the case of HPV infection purgation, the production of both investigated interleukins during the third trimester of pregnancy decreased compared to the concentrations of these interleukins during the first trimester of pregnancy (Fig. 3).

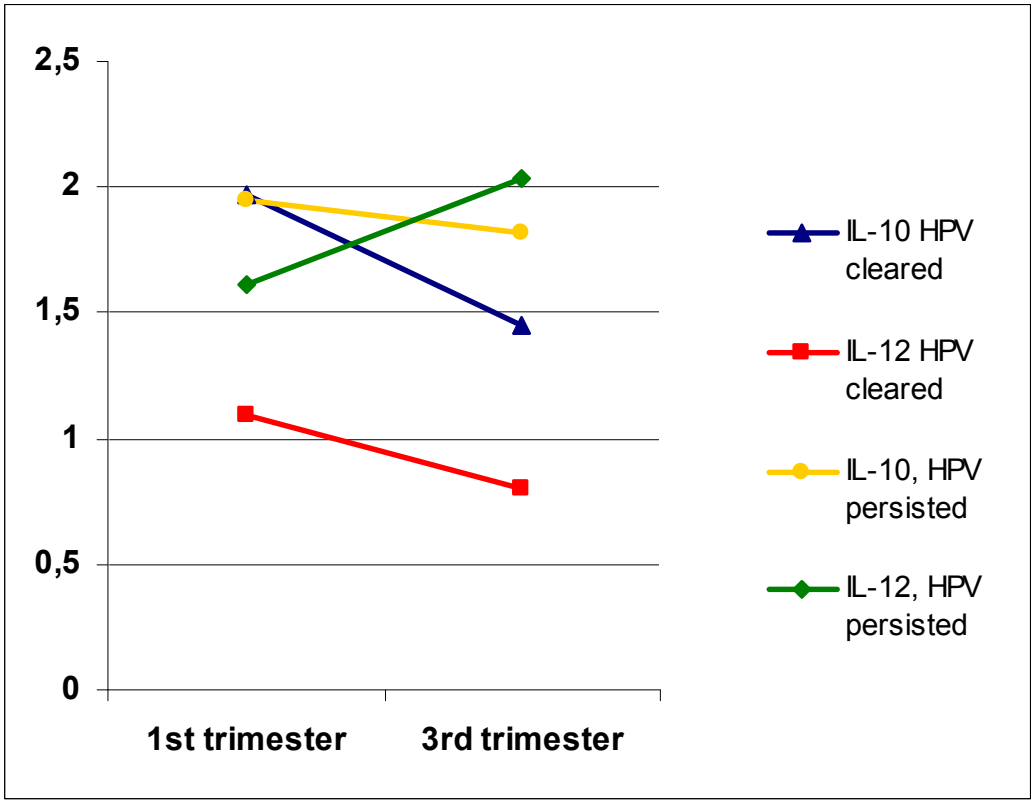


Figure 3. IL-10 and IL-12 in cases of HPV persistence and clearance.

The data we obtained show that in the case of HPV persistency during the third trimester of pregnancy, the Th1 cellular response was stimulated (IL-12 concentration was higher compared to the first trimester results), and the IL-10 level did not change (Th2 response). If the infection did not persist during the third trimester of pregnancy, the Th1 response was not stimulated and the IL-10 level in specimens decreased. Among HPV non-infected pregnant women the Th1 response activating the level of IL-12 during the first trimester of pregnancy was higher vs. the third trimester. The IL-10 concentration was very low during both trimesters of pregnancy. The low level of IL-10 could be influenced by numerous factors, which will be evaluated in further studies. It was determined that IL-12 stimulates cellular immunity as well as the production of Th1 mediators and inflammatory response, which are crucial components of antiviral protection.

Comparing investigated indicators of women who had persisted HPV infection in the cervix with non-infected women analogues, the indicators for former significantly higher IL-10 concentration (immunosuppressive impact) could stimulate the development of the HPV infection during the first trimester of pregnancy, and the IL-12 (stimulates antiviral protection influenced of Th1 functions) production during Th1 response activation was insufficient. When the infection was persistent, and during the third trimester of pregnancy the IL-10 level in fact was not alternated and the IL-12 concentration increased compared with the level during the first trimester of pregnancy, though it did not differ from HPV non-infected women, it means Th1 functions were active.

Our studies showed that in HPV non-infected women processes influenced by Th1 functions preponderate during pregnancy, and changes in IL-10 and IL-12 levels in cervicovaginal washing fluid are related to Th1 and Th2 local homeostasis. However, with HPV infection the varied cytokines synthesis response to pathogen emerged; it also was influenced by the hormone balance which varied during pregnancy. Thus, genital tract infections may impact the local secretion of interleukins, and the pregnancy may determine the even more multiform modulation of their production.

Conclusions

1. At the beginning of pregnancy (1st trimester), human papillomavirus (HPV) infection was detected for 17.8 % (95% CI = 13.25÷23.57) of pregnant women and at the end of pregnancy (3rd trimester) HPV was identified for 10.3 % (95% CI = 6.76÷17.16) of pregnant women; high oncogenic risk HPV types in the first trimester of pregnancy were identified for 57.7 % (95% CI = 38.92÷74.50) of HPV-positive women and at the end of pregnancy (3rd trimester) high risk HPV types were identified for 66.7 % (95% CI = 41.50÷85.04).
2. For 51.6 % (95% CI = 34.34÷66.03) of those infected in the first trimester, HPV was not determined in the third trimester of pregnancy, for 39.4 % (95% CI = 18.46÷49.97) the infection remained, and 4.2 % (95% CI = 1.54÷9.63) were infected during the pregnancy.
3. A decrease in the reduced glutathione (GSH) level and an increase in malonodialdehyde (MDA) level in the blood plasma during the development of the pregnancy confirmed the presence of oxidative stress. HPV infection was found not to affect this protective system.
4. Changes in interleukines (IL-10 and IL-12) concentrations in cervicovaginal washing fluid depend on HPV infection. When the infection persisted in the third trimester of pregnancy, T lymphocytes helpers 1 activation (IL-12 concentration) did not differ from non-infected women; though for non-persisting infection this response was weaker. The IL-10 concentration was higher (immunosuppressive effect) in investigated women groups with HPV infection vs. IL-10 concentration during the first and the third trimesters of pregnancy in non-infected women groups.

The Scientific Significance of the Study

- Established HPV infection in pregnant women and identified types of this virus enabled us to select high risk cervical cancer group.
- Infected pregnant women with high risk HPV type's needs additional screening after delivery in case of HPV persistence.
- It could be suggested to study the antioxidative system state during pathological pregnancy (maternal hypertensive state, preeclampsia, gestational diabetes) due to oxidative unbalance during pregnancy.
- It could be suggested to study changes in IL-10 and IL-12 concentrations in cervicovaginal washing fluid (local immunity) due to persistence of HPV infection.

SANTRAUKA

Darbo aktualumas

Žmogaus papilomos virusas (ŽPV) labiausiai pasaulyje paplitusi lytiniu keliu perduodama infekcija. Yra nustatyta, kad apie 70 proc. seksualiai aktyvios populiacijos susiduria su šia infekcija [1]. ŽPV paplitimo dažnis tarp nėščiųjų svyruoja nuo 5,5 iki 65,0 proc. [2]. Šį skirtumą gali sąlygoti įvairūs veiksniai, tarp kurių yra geografinis veiksnys, t.y. atskirose šalyse viruso paplitimas nėra vienodas. Todėl yra svarbu turėti kiekvienai šaliai būdingus ŽPV paplitimo duomenis.

Lietuvoje ŽPV paplitimo tyrimai pradėti apie 2000 metus. Nustatyta, kad tarp tirtų sveikų moterų (n=1120) apie vienas ketvirtadalis jų infekuotos didelės rizikos ŽPV. Dažniausiai tai jaunos ir mažesnį išsilavinimą turinčios moterys [3]. Apie gimdos kaklelio vėžio riziką, susijusią su ŽPV infekcija, liudija rezultatai, gauti tiriant sergančiąsias gimdos kaklelio vėžiu Lietuvoje [4]. Lietuvoje sergamumas gimdos kaklelio vėžiu yra vienas didžiausių Europoje [5]. Didelis ŽPV paplitimas, matyt, ir sąlygoja didelį sergamumą gimdos kaklelio vėžiu Lietuvoje bei mirtingumą nuo jo [6].

Apie nėščiųjų infekuotumą ŽPV pasaulyje duomenų nėra daug. Be to, kaip jau buvo minėta, tie duomenys nėra vienodi. Todėl svarbu nustatyti ŽPV paplitimą tarp nėščiųjų Lietuvoje ir palyginti gautus duomenis su esamais duomenimis kituose pasaulio regionuose.

Viena vertus, nėštumo metu vykstantys fiziologiniai procesai moduliuoja apsaugines organizmo sistemų (imuninės ir antioksidacinės) funkcijas ir gali didinti infekuotumo ŽPV riziką bei skatinti viruso išlikimą gimdos kaklelio epitelio ląstelėse ir infekcijos progresavimą, antra vertus, ŽPV infekcija taip pat gali įtakoti minėtų sistemų veiklą. Todėl svarbu nustatyti ne tik nėščiųjų infekuotumą ŽPV, bet ir įvertinti šios infekcijos įtaką minėtoms organizmo apsauginėms sistemoms. Juolab, kad yra duomenų, jog oksidacinis stresas, besiformuojantis nėštumo metu, gali sąlygoti įvairias nėštumo komplikacijas. Gilėjant oksidaciniam stresui nėštumo metu vystosi nėščiųjų hipertenzinės būklės, preeklampsija, gestacinis diabetas [7, 8]. Oksidacinis stresas svarbus ir vaisiaus vystymuisi [9].

Citokinų produkcijos (koncentracijos) pokyčiai bei jų funkcinis aktyvumas yra svarbūs veiksniai vertinant vietinio imuniteto dinamiką nėštumo metu. Duomenų apie interleukinų koncentracijos pokyčius ir sąsajas su ŽPV infekcija nėštumo metu neradome. Pagal sekretuojančių citokinų pobūdį imuninį atsaką reguliuojantys T limfocitai-helperiai (Th) skirstomi į du potipius (Th1 ir Th2), kurie slopina vienas kito aktyvumą. Interleukinas-12 (IL-

12) yra vienas iš pagrindinių veiksnių, skatinančių Th1 atsaką, o produkuojamas Th2 limfocitų interleukinas-10 (IL-10) šį atsaką slopina. Kai yra virusinė infekcija, atpažįstant ir naikinant virusų pažeistas ląsteles ypač svarbios Th1 limfocitų funkcijos ir jų sintetinami citokinai, todėl svarbu tyrinėti šių interleukinų pokyčius nėščiųjų organizme priklausomai nuo infekuotumo ŽPV.

Darbo tikslas

Nustatyti ŽPV infekcijos įtaką nėščiosios organizmo antioksidacinės sistemos būklei bei įvertinti vietinio imuniteto atsaką į šią infekciją.

Darbo uždaviniai

1. Nustatyti tiriamųjų infekuotumą ŽPV ir ŽPV, kuriuo jos infekuotos, tipą bei palyginti tiriamųjų infekuotumą ŽPV esant pirmajam ir trečiajam jų nėštumo trimestrui;
2. Ištirti antioksidacinės sistemos būklę nėštumo metu priklausomai nuo ŽPV infekcijos;
3. Ištirti vietinį imunitetą rodančių interleukinų (IL-10 ir IL-12) koncentracijų pokyčius nėštumo metu priklausomai nuo ŽPV infekcijos.

Darbo naujumas

Atsižvelgiant į tai, kad Lietuvoje yra didelis sergamumas gimdos kaklelio vėžiu bei mirtingumas nuo jo, mūsų nustatytas nėščiųjų infekuotumas ŽPV bei identifikuoti jo tipai leidžia įvertinti infekuotumo ŽPV pokyčius nėštumo metu didelės gimdos kaklelio vėžio rizikos populiacijoje ir sudaro galimybę palyginti gautus duomenis su kitų šalių autorių gautais duomenimis.

Įrodyta, kad nėštumo metu moterims vystosi oksidacinis stresas, kurio lygis nepriklauso nuo infekuotumo ŽPV.

Nustatyta, kad IL-10 ir IL-12 koncentracijos pokyčiai gimdos kaklelio nuoplovose (vietinis imunitetas) susiję su Th1 ir Th2 limfocitų funkcija ir priklauso nuo infekuotumo ŽPV.

Rezultatai

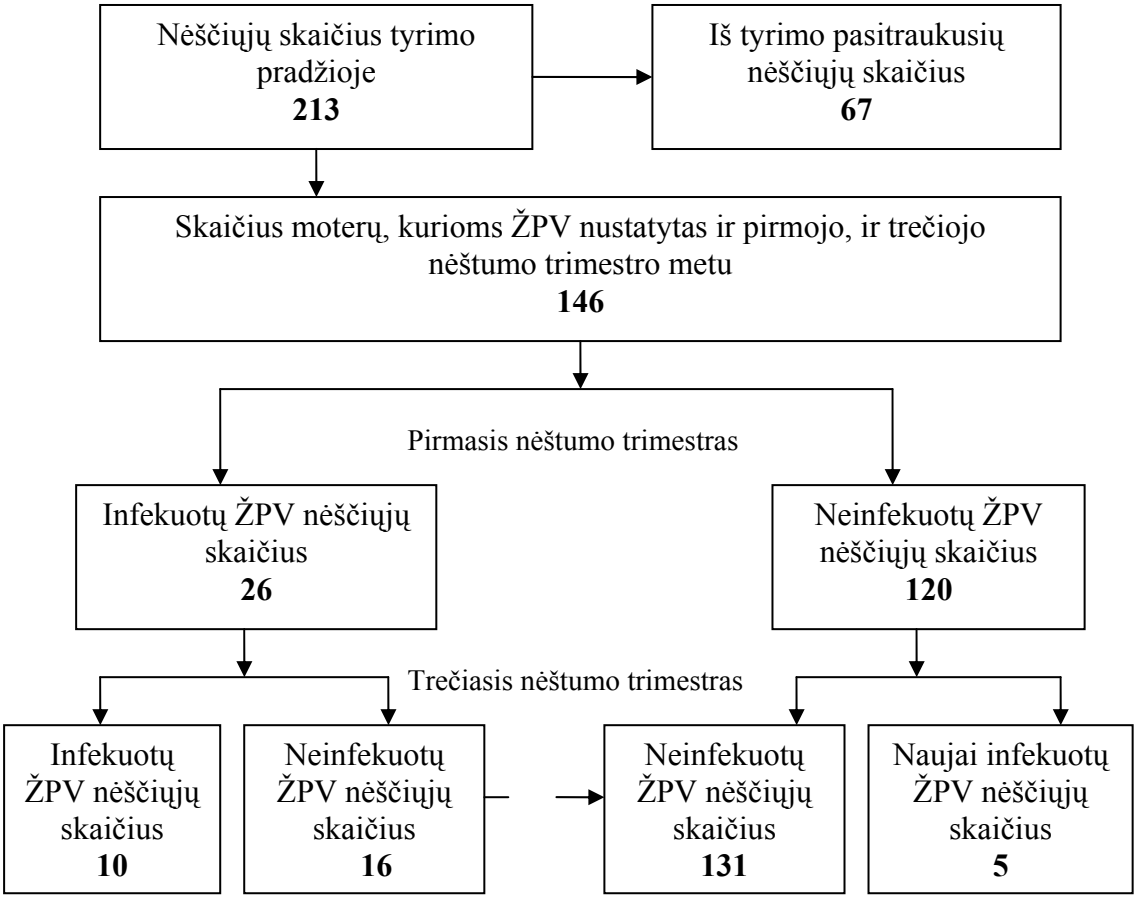
Bendrosios charakteristikos. Į tyrimą įtraukta 213 nėščiųjų, sutikusių dalyvauti atliekant tyrimą ir pasirašiusių Informuoto asmens sutikimo formą. Tarp į tyrimą įtrauktų 213 nėščių moterų 76,5 proc. (n=163) sudarė ištekėjusios, 58,7 proc. (n=125) - tarnautojos, 77,5 proc. (n=165) - turinčios aukštąjį išsilavinimą; didžiausia dalis moterų buvo lietuvės - 81,2 proc. (n=173). Pirmuosius lytinius santykius iki 18 metų turėjo 30,5 proc. (n=65) nėščiųjų. Apie pusė į tyrimą įtrauktų moterų (55,4 proc., n=116) turėjo nuo 1 iki 2 lytinių partnerių. Pagal kontraceptinių priemonių naudojimą moterys pasiskirstė taip: 52,6 proc. (n=112) moterų naudojo hormonines ir 39,9 proc. (n=85) - kitas priemones. Ginekologinėmis ligomis buvo sirgusios 69,5 proc. (n=148) moterų.

Nėščiųjų infekuotumas ŽPV bei atskirais jo tipais

Tyrimo pradžioje (pirmasis nėštumo trimestras) tarp 213 nėščiųjų ŽPV nustatytas 17,8 proc. (n=38) moterų (95% PI = 13.25÷23.57). Tarp identifikuotų tipų ŽPV 7,8 proc. nėščiųjų nustatyti 16 ir 18 tipų ŽPV, 21,1 proc. nėščiųjų - 31, 52 ir 58 tipų ŽPV, 10,5 proc. nėščiųjų - 33, 39, 51 ir 56 tipų ŽPV ir 13,2 proc. konstatuota kelių tipų ŽPV; likusią dalį - 47,4 proc. sudarė neidentifikuotų tipų ŽPV.

Į pakartotinį ŽPV tyrimą trečiąjį nėštumo trimestrą 67 moterys neatvyko: pakeitė gydymo įstaigą, įvyko persileidimas, prieš laiką pagimdė. Pažymėtina, kad tarp jų 14 nėščiųjų pirmąjį nėštumo trimestrą buvo nustatyta ŽPV infekcija. Taigi trečiąjį nėštumo trimestrą buvo tirtos 146 nėščiosios, tarp kurių ŽPV nustatytas 10,3 proc. (n=15) (95 % PI=6,76÷17,16). Tarp identifikuotų tipų ŽPV 16 ir 18 tipai nustatyti atitinkamai 26,6 proc. nėščiųjų, 58 tipas - 20,0 proc. ir 20,0 proc. nėščiųjų - kelių tipų ŽPV; likusią dalį - 33,4 proc. sudarė neidentifikuotų tipų ŽPV infekuotos nėščiosios.

Infekuotumo ŽPV pokyčiai priklausomai nuo nėštumo trimestro. Siekiant palyginti infekuotumo ŽPV pokyčius priklausomai nuo nėštumo trimestro, į duomenų apskaičiavimą įtrauktos tik tos nėščiosios, kurioms ŽPV nustatytas ir pirmojo, ir trečiojo trimestrų metu. Tokių moterų buvo 146, tarp kurių 17,8 proc. (n=26) buvo infekuotos ŽPV (95 % PI=12,4÷24,87). Trečiojo nėštumo trimestro metu ŽPV nustatytas 15 nėščiųjų, tarp kurių 10 moterų ŽPV persistavo, 5 moterys užsikrėtė nėštumo metu ir 16 moterų, kurioms ŽPV testas buvo teigiamas pirmajame nėštumo trimestre, ŽPV infekcija išnyko (1 pav.).



1 pav. Moterų infekuotumo ŽPV pokyčiai nėštumo metu

Infekuotumas ŽPV pagal atskirus viruso tipus pateiktas 1 lentelėje.

1 lentelė. ŽPV tipų pokyčiai nėštumo metu

Grupė	ŽPV tipas	Nėščiųjų skaičius (n)		
		Pirmasis nėštumo trimestras	Trečiasis nėštumo trimestras	
			infekcija persistuoja	naujas užsikrėtimas
I	16	2	1	1
II	18	1	1	1
III	31 52 58	2 3 1	2 (58 tipas)	1 (58 tipas)
IV	39 51 56	1 1 1	0	0
V	Kelių tipų ŽPV:	3	2	1 (16, 18 ir 31 tipai)
	16 ir 45 31 ir 51 52 ir 36	1 1 1	1 1 0	
VI	Neidentifikuotas tipas	11	4	1
Iš viso		26	10	5

Trečiajame nėštumo trimestre tarp persistuojančios ŽPV infekcijos buvo 16, 18, 58 bei kelių (16 ir 45; 31 ir 51) tipų ŽPV infekcija, tarp moterų, kurios užsikrėtė nėštumo metu, identifikuota 16, 18 ir 58 tipų ŽPV bei mišri infekcija (16, 18 ir 31 tipų ŽPV). Persistuoti linkę didelės onkogeninės rizikos ŽPV tipai. Taigi moterys, kurioms nėštumo metu nustatyta didelės rizikos tipų ŽPV, būtina stebėti ir po gimdymo.

Nėščiųjų rizika užsikrėsti ŽPV. Analizuota rizika užsikrėsti ŽPV priklausomai nuo nėščiosios amžiaus, lytinių partnerių skaičiaus, persirgtų ginekologinių ligų bei kitų veiksnių. Didesnė rizika užsikrėsti ŽPV nustatyta sirgusioms ginekologinėmis ligomis nėščiosioms (ŠS=0,383; 95 % PI=0,222÷0,659; p=0,013) ir tendencija - jaunesnėms nei 29 metų moterims (ŠS=0,291; 95 % PI=0,186÷0,456; p=0,063). Tarp ginekologinių ligų - dažniausia buvo dubens uždegiminė liga.

ŽPV infekcijos įtaka nėščiųjų antioksidacinės sistemos būklei ir lipidų peroksidacijos intensyvumui

Įvertintas moterų antioksidacinės sistemos pajėgumas nėštumo metu. Atlikti kraujo plazmos ir gimdos kaklelio nuoplovų tyrimai (2 lent.).

2 lentelė. Antioksidacinės sistemos būklės ir lipidų peroksidacijos intensyvumo pokyčiai tirtų moterų kraujo plazmoje ir gimdos kaklelio nuoplovose nėštumo metu

Rodiklis	Trimestras	Vidurkis	N	Standartinis nuokrypis	Vilkoksono kriterijus	Reikšmingumas
Kraujo plazma						
GSH	I	0.50	60	0.21	-5.30	0.00
	III	0.28	60	0.12		
GSSG	I	0.29	60	0.13	-6.24	0.00
	III	0.13	60	0.05		
GSH+GSSG	I	0.79	60	0.28	-6.00	0.00
	III	0.42	60	0.14		
GSH/GSSG	I	2.33	60	2.60	-1.82	0.07
	III	2.34	60	1.29		
BAA	I	30.96	62	10.78	-2.51	0.01
	III	27.45	62	9.12		
MDA	I	7.12	62	3.05	-5.97	0.00
	III	12.62	62	4.34		
Gimdos kaklelio nuoplovos						
GSH	I	0.60	65	0.53	-3.94	0.00
	III	0.32	65	0.12		
GSSG	I	0.27	65	0.15	-1.55	0.12
	III	0.21	65	0.10		
GSH+GSSG	I	0.87	65	0.62	-3.91	0.00
	III	0.53	65	0.17		
GSH/GSSG	I	2.38	65	1.44	-1.72	0.09
	III	2.00	65	1.33		
BAA	I	23.38	64	9.60	-1.63	0.10
	III	26.28	64	10.54		
MDA	I	4.31	65	4.02	-1.76	0.08
	III	3.54	65	5.27		

GSH - redukuotas glutationas (µmol/ml), GSSG - oksiduotas glutationas (µmol/ml), GSH+GSSG - bendrasis glutationas (µmol/ml), GSH/GSSG - glutationo formų santykis, BAA - bendrasis antioksidacinis aktyvumas (%), MDA - malono dialdehidas (nmol/ml).

Iš 2 lentelėje pateiktų duomenų matyti, kad daugumos tirtųjų rodiklių reikšmės (redukuoto ir oksiduoto glutationo, bendrasis oksidacinis aktyvumas, malono dialdehido kiekis), nustatytos tiriant kraujo plazmą statistiškai reikšmingai skiriasi tarp pirmojo ir trečiojo nėštumo trimestrų. Nėščiųjų kraujo plazmoje trečiajame nėštumo trimestre sumažėjo abiejų glutationo formų bei padidėjo malono dialdehido koncentracija, gimdos kaklelio nuoplovose nėštumo metu statistiškai reikšmingai pakito redukuotojo ir bendrojo glutationo kiekis. Statistiškai reikšmingas glutationo formų aktyvumo sumažėjimas bei padidėjusi malono dialdehido koncentracija rodo, kad nėštumo metu formuojasi oksidacinis stresas.

Toliau palyginta, kaip kinta antioksidacinės sistemos būklė nėštumo metu atskirai infekuotoms ir neinfekuotoms moterims. Analizuojant antioksidacinės sistemos būklės ir lipidų peroksidacijos intensyvumo pokyčius, nustatytus tiriant ŽPV infekuotų ir neinfekuotų moterų kraujo plazmą, bendrasis antioksidacinis aktyvumas statistiškai reikšmingai nesiskyrė tarp pirmojo ir trečiojo nėštumo trimestrų tiriant ir infekuotų, ir neinfekuotų moterų kraujo plazmą.

Tiriant antioksidacinės sistemos būklę atspindinčių rodiklių pokyčius nėščiųjų gimdos kaklelio nuoplovose statistiškai reikšmingai sumažėjo tik glutationo (redukuoto ir bendrojo) koncentracija ir ŽPV infekuotų, ir neinfekuotų nėščiųjų grupėse. Tai leidžia manyti, kad gimdos kaklelis nėštumo metu yra geriau apsaugotas nuo laisvųjų radikalų poveikio.

Nagrinėjant antioksidacinės sistemos būklės pokyčius nėštumo metu pagal kraujo plazmos rodiklius tarp ŽPV infekuotų ir neinfekuotų moterų pirmojo nėštumo trimestro metu nustatyta ŽPV infekuotų moterų mažesnė oksiduoto glutationo koncentracija bei mažesnis redukuoto ir oksiduoto glutationo santykis. Pagal gimdos kaklelio nuoplovų tyrimo rezultatus statistiškai reikšmingo skirtumo nenustatyta (3 lent.).

3 lentelė. Antioksidacinės sistemos būklės ir lipidų peroksdacijos intensyvumo pokyčiai kraujo plazmoje ir gimdos kaklelio nuoplovose

Rodiklis	Vidurkis	Standartinis nuokrypis	Vidurkis	Standartinis nuokrypis	Vilkoksono kriterijus	Reikšmingumas
	ŽPV neinfekuotos		ŽPV infekuotos			
Kraujo plazma						
GSH1	0.49	0.23	0.55	0.20	1381.5	0.14
GSH2	0.27	0.11	0.29	0.14	1296.5	0.96
GSSG1	0.32	0.13	0.23	0.11	1096	0.01
GSSG2	0.13	0.05	0.14	0.08	1294.5	0.94
GSH+GSSG1	0.81	0.31	0.78	0.24	1500	0.83
GSH+GSSG2	0.41	0.14	0.48	0.33	1270.5	0.68
GSH1/GSSG1	1.76	1.33	3.96	4.78	1212	0.00
GSH2/GSSG2	2.25	1.18	3.01	3.14	1214.5	0.24
MDA1	6.65	2.39	8.01	3.55	1447.5	0.19
MDA2	12.61	4.39	13.40	5.32	1278	0.76
BAA1	30.86	9.59	31.49	11.67	1553	0.79
BAA2	27.86	9.37	26.25	8.87	694	0.55
Gimdos kaklelio nuoplovos						
GSH1	0.59	0.59	0.67	0.58	1564.5	0.73
GSH2	0.33	0.12	0.30	0.13	821.5	0.28
GSSG1	0.27	0.16	0.26	0.15	1524	0.72
GSSG2	0.22	0.10	0.22	0.11	895	0.90
GSH+GSSG1	0.86	0.69	0.93	0.69	1581	0.85
GSH+GSSG2	0.55	0.18	0.66	0.58	898.5	0.94
GSH1/GSSG1	2.17	1.31	2.58	1.57	1466.5	0.19
GSH2/GSSG2	1.96	1.12	2.07	1.60	867.5	0.63
MDA1	3.83	3.69	4.40	4.15	1574.5	0.66
MDA2	2.84	2.56	5.60	8.86	1216	0.34
GAA1	24.08	9.43	23.83	10.19	1612	0.94
GAA2	27.84	10.44	23.96	10.64	680	0.21

GSH – redukuotas glutationas (µmol/ml), GSSG – oksiduotas glutationas (µmol/ml), GSH+GSSG – bendrasis glutationas (µmol/ml), GSH/GSSG – glutationo formų santykis, MDA – malono dialdehidas (nmol/ml), BAA bendrasis antioksidacinis aktyvumas (%), 1 – pirmasis nėštumo trimestras, 2- trečiasis nėštumo trimestras.

Nepaisant to, kad nėščiųjų kraujo plazmoje statistiškai reikšmingai padidėjo malono dialdehido koncentracija trečiajame nėštumo trimestre ir ŽPV infekuotų, ir neinfekuotų moterų grupėse, visgi lyginant tarpusavyje ŽPV infekuotas ir neinfekuotas nėščiąsias statistiškai reikšmingo skirtumo nėra. Šis lipidų peroksidacijos reakcijos produktas rodo oksidacinį stresą nėštumo metu, tačiau ŽPV infekcija neturi įtakos jo koncentracijai.

ŽPV infekcijos įtaka vietinio imuniteto atsakui

Siekiant įvertinti, ar ŽPV infekcija turi įtakos nėščiųjų vietinio imuninės sistemos atsakui į šią infekciją, palygintos tirtų interleukinų koncentracijos tiek pirmojo, tiek trečiojo nėštumo trimestrų metu tarp infekuotų ir neinfekuotų ŽPV nėščiųjų (4 lent.). Nustatyta, kad infekuotų moterų gimdos kaklelio nuoplovose pirmojo nėštumo trimestro metu IL-10 koncentracija buvo statistiškai reikšmingai didesnė, palyginti su šio interleukino koncentracija neinfekuotų ŽPV moterų gimdos kaklelio nuoplovose, o trečiojo nėštumo trimestro metu neinfekuotų nėščiųjų grupėje IL-10 visai nenustatyta. Taigi IL-10 koncentracijos skirtumai tiek pirmajame, tiek trečiajame nėštumo trimestre tarp infekuotų ir neinfekuotų ŽPV yra ženklūs. Tai rodo, kad infekuotų moterų buvo suaktyvinta Th2 limfocitų (supresuojančio ląstelinio imuniteto atsaką) funkcija.

4 lentelė. IL-10 ir IL-12 koncentracijų palyginimas tarp infekuotų ir neinfekuotų ŽPV nėščių moterų

Nėštumo trimestras	Rodikli s	ŽPV nustatytas			ŽPV nenustatytas			Vilkokson o kriterijus	Reikšminguma s
		n	vidurki s	standartini s nuokrypis	n	vidurki s	standartini s nuokrypis		
I	IL-10 (pg/ml)	10	1.95	1.64	28	0.01	0.00	450	0.00
	IL-12 (pg/ml)	9	1.61	1.76	20	2.47	2.21	111	0.26
III	IL-10 (pg/ml)	10	1.82	1.43	28	0.00	0.00	443	0.00
	IL-12 (pg/ml)	9	2.04	2.67	20	1.93	2.13	134	0.96

Išvados

1. Tyrimo pradžioje (pirmasis nėštumo trimestras) tiriamųjų infekuotumas ŽPV siekė 17,8 proc. (95% PI=13,25÷23,57), tyrimo pabaigoje (trečiasis nėštumo trimestras) - 10,3 proc. nėščiųjų (95% PI=6,76÷17,16); didelės onkogeninės rizikos ŽPV tipai pirmajame nėštumo trimestre nustatyti 57,7 proc. (95% PI = 38,92÷74,50) ŽPV infekuotų tiriamųjų , o trečiajame - 66,7 proc. (95% PI = 41,50÷85,04).
2. Nėštumo metu savaime pasveiko 51,6 proc. (95% PI = 34,34÷66,03) ŽPV infekuotų tiriamųjų; 39,4 proc. (95% PI = 18,46÷49,97) infekcija išliko; nėštumo metu ŽPV užsikrėtė 4,2 proc. (95% PI = 1,54÷9,63) tiriamųjų.
3. Statistiškai reikšmingas glutationo formų koncentracijos sumažėjimas bei padidėjusi malono dialdehido koncentracija kraujo plazmoje rodo, kad nėštumo metu formuojasi oksidacinis stresas. ŽPV infekcijos įtakos šio streso lygiui nenustatyta.
4. Nėštumo metu esant ŽPV infekcijai pakito interleukinų (IL-10 ir IL-12) gamyba. Persistuojant infekcijai trečiajame nėštumo trimestre IL-12 koncentracija nesiskyrė nuo neinfekuotų nėščiųjų, o infekcijai išnykus IL-10 koncentracija buvo didesnė (imunosupresinis poveikis) tirtų infekuotų ŽPV nėščiųjų grupėje, palyginti su IL-10 koncentracija tirtų neinfekuotų tiriamųjų grupėje.

Darbo praktinė reikšmė

- Nustatyti ŽPV paplitimą tarp nėščiųjų ir identifikuoti jo tipus būtų naudinga vertinant ŽPV paplitimą Lietuvoje bei atrenkant grupes moterų, kurių rizika susirgti gimdos kaklelio vėžiu didelė.
- Moterys, nėštumo metu infekuotos didelės onkogeninės rizikos tipų ŽPV, turi būti sekamos dėl ŽPV po gimdymo, nes nėštumas gali sąlygoti ŽPV infekcijos persistavimą.
- Nepaisant to, kad ŽPV infekcijos įtakos oksidacinio streso lygiui nenustatyta, visgi įvertinti antioksidacinės sistemos būklę būtų naudinga esant nėščiųjų hipertenzinėms būklėms, gestaciniam diabetui, kadangi nėštumo metu oksidacinis stresas gilėja.
- Interleukinų (IL-10 ir IL-12) koncentracijos tyrimai gali būti vertingi prognozuoti didelės rizikos tipų ŽPV infekcijos persistavimą po gimdymo.

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