ACTA MEDICA LITUANICA. 2010. Vol. 17. No. 3–4. P. 83–92
DOI: 10.2478/v10140-010-0011-y
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The status of antioxidative system in pregnant women infected with human papilloma virus

Birutė Surinėnaitė[1],

Gintautas Domža[2],

Janina Didžiapetrienė[1, 2],

Gražina Prasmickienė[1],

Živilė Gudlevičienė[1],

Audronė Jakaitienė[3]

[1] *Institute of Oncology, Vilnius University, Vilnius, Lithuania*

[2] *Vilnius University, Vilnius, Lithuania*

[3] *Institute of Mathematics and Informatics, Vilnius University, Vilnius, Lithuania*

Background. The concentration of reduced (GSH), oxidized (GSSG) and total (GSH + GSSG) glutathione, the level of malondialdehyde (MDA), and the general antioxidant activity (GAA) was determined in blood plasma and cervicovaginal washing fluid of pregnant women infected and non-infected with human papilloma virus (HPV).

Materials and methods. The population of 213 pregnant women visiting one of the Centers of the Central Outpatient Clinic of Vilnius (Lithuania) in 2008–2010 was included in the study. These women were examined for HPV infection and its type. The tests were performed in the first and third trimesters of pregnancy in blood plasma and cervicovaginal washing fluid. The MDA level was tested by the thiobarbituric acid assay. The amount of glutathione forms was determined using a recycling system with 5,5'-dithiobis 2-nitrobenzoic acid and glutathione reductase. General antioxidant activity (GAA) was measured by Tween 80 oxidation in a specific test. All parameters were analyzed spectrophotometrically.

Results. The concentration of all glutathione forms and GAA reduced during pregnancy, while the MDA level was enhanced in the blood plasma of all women included in the analysis. Only two of these parameters changed in the cervicovaginal washing fluid. No significant change of GAA was determined comparing groups of women while considering HPV infection. The GSH / GSSG ratio significantly increased in HPV-negative and decreased in HPV-positive women. While comparing HPV-positive and HPV-negative women of similar age, no significant difference of any parameter was found in cervicovaginal washing fluid and in blood plasma in the third trimester. In the first trimester the GSSG level was significantly lower, the GSH level was higher, and the GSH / GSSG ratio was twice higher in HPV-infected women. All pregnant women were compared with non-pregnant women to confirm the influence of pregnancy on the MDA level. In the first trimester, this parameter for pregnant women was close to that of non-pregnant women, while with the developing pregnancy the MDA level increased 1.6 times. No significant difference in the parameter was determined for pregnant women with HPV infection.

Conclusions. A decrease of GSH concentration and an increase of MDA level in blood plasma with the development of pregnancy confirmed the presence of a general oxidative stress. The lower levels of GSH and GSH + GSSG in the cervicovaginal washing fluid, if to compare the two trimesters of pregnancy, can be considered as markers of a local oxidative stress. HPV infection depressed the antioxidative system in general and did not affect it at the local level. Systemic oxidative stress, rather than HPV infection, influenced the lipid peroxidation process during pregnancy. The level of MDA is recommended to be tested also during a normal pregnancy, although this parameter should not be considered as an additional biomarker of cervical carcinoma risk. Changes of the antioxidative system variables could induce a deep oxidative imbalance during a pathological pregnancy, and oxidative stress might cause a persistent HPV infection, suggesting the importance of the additional screening of HPV-infected women after delivery.

Key words: HPV infection, pregnancy, antioxidative system

Correspondence to: Birutė Surinėnaitė, Institute of Oncology, Vilnius University, Scientific Research Center, Baublio Str. 3B, LT-08406 Vilnius, Lithuania. E-mail: birute.surinenaite@vuoi.lt

INTRODUCTION

Human papilloma virus (HPV) is the most common sexually transmitted infection in the world. About 70% of sexually active population confront this infection (1).

The physiological changes during pregnancy modulate the functions of essential protective systems (immune and antioxidative); consequently, HPV infection might affect a female organism in a specific manner in this period (2). The rate of HPV prevalence among pregnant women has been found to range from 5.5 to 65.0% (3). Moreover, pregnant women are at a higher risk to be infected with HPV, particularly with high-risk HPV types associated with cervical cancer (4, 5). In most cases, HPV is known as one of the main risk factors of cervical cancer. Therefore, it is important to detect the infection during pregnancy and to determine the status of the antioxidative system in order to prevent the risk of cervical cancer and to enhance the level of systemic protection of the organism (6).

Oxidative stress as a long-lasting abnormal excess of reactive oxygen species (ROS) can also be suggested as one of the main factors of cancer risk, including cervical cancer (7). The level of antioxidant compounds in blood plasma can modulate the progression of a latent HPV infection to sub-clinical lesions. Also, increased systemic oxidative stress contributes to a variety of maternal complications during pregnancy (8). Markers of the stress are characteristic of women to whom the development of preeclampsia is determined during pregnancy (9–11). However, in general, the contribution of oxidative stress to the initiation of the disease remains to be an interrogation note (12). Furthermore, systemic oxidative stress was also shown to be enhanced during pregnancies complicated by maternal diabetes (13) or intrauterine growth retardation (14). Dynamical changes of lipid peroxidation indices, of certain antioxidant compounds and of antioxidative enzyme activities during pregnancy and differences of parameters due to virus infection could help to predict a normal or abnormal course of pregnancy (15). Malondialdehyde (MDA) level is a sensitive parameter of lipid peroxidation (16). The concentration of glutathione is an important antioxidant index as antioxidant defence is regulated by the redox pair of GSH / GSSG. The general antioxidant activity (GAA) shows the total status of the antioxidative capacity of an organism (17, 18). The effect of HPV infection on the antioxidative system, indicated by the parameters mentioned above, has not yet been examined in the context of pregnancy. Moreover, no data were found on the differences in antioxidative system parameters on the general (blood) and local (cervix) levels in HPV-infected persons. Consequently, it is important to examine these parameters on both levels.

The goal of the present research was to investigate the concentration of reduced (GSH), oxidized (GSSG) and total (GSH + GSSG) glutathione, malondialdehyde (MDA) level, and general antioxidant activity (GAA) in blood plasma and cervicovaginal washing fluid of pregnant women and to evaluate the status of the antioxidative system in HPV-infected and non-infected persons.

MATERIALS AND METHODS

Population. A population of 213 pregnant women attending one of the Centers of the Central Outpatient Clinic of Vilnius (Lithuania) in 2008–2010 was included in the study. The age of the women was 20–40 years. The women were examined for HPV infection and its type. Tests were performed on the first and the third trimesters of pregnancy. The women were asked to complete a questionnaire of 22 questions. The questions concerned social demographic features (age, education, marital status, nationality, social status), sexual behaviour (first sexual experience, the number of sexual partners, the non-marital sexual contacts of the partner), and about gynaecological history (number of deliveries and abortions, first mensis, gynaecological diseases in the past, use of contraception, results of cytological cervical smears). The study was performed with the permission of the Lithuanian Bioethics Committee (26-03-2008, No 21/1).

HPV identification and typing. HPV infection was detected in cervicovaginal washing fluid. During gynaecological examination, the cervix was washed with 10 ml of saline. The material was collected in a sterile container which 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 –20 °C and was prepared for testing in a few stages. First, DNA sample was extracted using a commercial *Sorpoclean* DNA extraction kit ("SORPO diagnostics", Lithuania) following the manufacturer's recommendations.

Considering the published data (19), HPV types were grouped into several categories: group I included HPV of type 16, which determines about 54.6% of cervical cancer cases; group II included HPV of type 18 (18.8% of cases); group III contained HPV of types 31, 52, and 58 (1.1–4.4% of cases), group IV consisted of types 33, 39, 51, and 56 (0.2–0.8% of cases); group V included several HPV types, and group VI contained HPV of unidentified types.

Polymerase chain reaction (PCR). PCR was performed to identify HPV, using 50 µl of a solution containing 45 µl of commercial *HPV Master Mix* solution ("SORPO diagnostics") and 5 µl of DNA sample in accordance with the manufacturer's recommendations. DNA was examined to ensure the presence of ß globin gene in all samples prior to PCR for HPV identification as described in (20). Each PCR analysis to identify HPV was performed using a positive control from the commercial kit. Samples without DNA (with de-ionized water) were used as a negative control. HPV-positive samples were further examined: an additional PCR was 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 ("SORPO diagnostics").

Visualization of 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 the products were analyzed in a UV transilluminator (320 nm). Pictures of the results were taken and stored in hardware.

Analysis of antioxidative system variables. Antioxidative system variables were determined in two samples of blood plasma and cervicovaginal fluid collected in the first and third trimesters of pregnancy, respectively. The level of the lipid peroxidation product MDA (nmol/ml) was determined by thiobarbituric acid (TBA) assay based on the release of color MDA / TBA complexes as described in (21). The concentration of functional antioxidant glutathione (mkmol/ml), both in reduced (GSH) and oxidized (GSSG) forms, was detected using a recycling system with 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) and glutathione reductase (GR), following the method of (22). Analytes were pre-treated with 2-vinylpiridine to determine the concentration of GSSG. The GSH / GSSG ratio was calculated. GAA (%) was determined in the Tween 80 oxidation test. 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 at 40 °C. The cooled reaction mixture was then added to 1 ml of 40% trichloracetic acid and was stored for 1 h at room temperature. The mixture was centrifuged for 15 min at 8 000 rpm, the supernatant was removed to 2 ml of 0.25% TBA, and the mixture was heated for 15 min. The cool mixture was then analysed for absorption at 532 nm, indicating the amount of MDA. All parameters were analyzed spectrophotometrically. A SF-46 LOMO spectrophotometer was used to measure the optical density.

Statistical analysis. The statistical analysis was performed using the SPSS (v.17) and Microsoft Office Excel 2007 statistical software package. All variables under analysis were qualitative. As almost 2/3 of data did not pass the normality test, the differences between the 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 independent and for dependent samples, respectively. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

The increased systemic oxidative stress in pregnant women should be considered as a result of physiological factors contributing to the development of fetus (23). Moreover, virus infections usually also enhance the oxidative stress (15). Changes of the variables showing the status of the protective antioxidative system during pregnancy and due to virus infection could help to predict the course of pregnancy as mentioned above (24). Consequently, differences in the antioxidative system variables were examined in early and late trimesters of pregnancy on the organism (blood plasma) and local (cervicovaginal washing fluid) levels considering also HPV infection.

General aspects. The study cohort comprises 213 pregnant women who signed the informed consent form; 76.5% (n = 163) of the women were married; 58.7% (n = 125) were officials, 77.5% (n = 165) had a higher education; the majority of women were Lithuanians (81.2%, n = 173); 30.5% (n = 65) had the first sexual relationship at the age of less than 18 years. About half of the participants (55.4%, n = 116) had one or two partners; 52.6% (n = 112) used hormonal contraceptives and 39.9% (n = 85) used other contraceptive methods. Anamnesis of a gynaecological disease was present in 69.5% (n = 148) of women. Four women had cervical conization before pregnancy due to cervical intraepithelial changes.

HPV infection and HPV types. In 17.8% (n = 38) of the women, HPV infection was found in the first trimester of pregnancy; 13.2% carried HPV of types 16 and 18 or of type 16 combined with other HPV types; 21.1% carried HPV of types 31, 52, and 58. These HPV types, according to reference (25), determine 1.1% to 4.4% cases of cervical cancer. HPV of types 33, 39, 51 and 56 was found in 10.5% of the women. These HPV types determine 0.2 to 0.8% cases of cervical cancer. The HPV type for the rest of women (47.4%) was not identified. During the third trimester of pregnancy, 67 women did not repeat examination of cervical outwash for HPV due to changed location, miscarriage or preterm delivery; 14 of them carried HPV infection in the first trimester of pregnancy. Therefore, 146 women were studied in the third trimester of pregnancy, including 11% (n = 16) (95%, CI = 6.76–17.16) of women to whom HPV infection was identified. The identified spectrum of HPV types in the third trimester of pregnancy was narrower compared to the analogical spectrum in the first trimester of pregnancy; however, the percentage of women infected with HPV of types 16 and 18 was significantly higher (respectively 13.2% in the first trimester and 13.8% in the third trimester of pregnancy).

Antioxidative system variables. The analysis was started by comparing antioxidative system variables for all women (both HPV-infected and non-infected) in the first and the third trimesters of pregnancy. The results are presented in Table 1.

A significant difference was determined for most variables of the antioxidative system (concentration of reduced, oxidized and total glutathione, amount of malondialdehyde, and general antioxidative activity) in the blood plasma of a pregnant woman while comparing trimesters of the pregnancy. The concentration of glutathione forms and GAA was reduced during pregnancy, while the MDA level was enhanced. On the other hand, only the average level for two of these variables (amount of reduced and total glutathione) changed significantly during the development of pregnancy in the analyses of cervicovaginal washing fluid.

Table 1. **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. 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 (%)**

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		

Here, it should be noted that the organism level (blood plasma) showed the status of the antioxidative system better than the local level (cervicovaginal washing fluid), even taking into account that different values of each parameter were detected when comparing both fluids. The reason might be that the cervix is possibly better protected from changes of essential functions than the whole body during pregnancy. A significant drop of the average GSH concentration and an increase of the average MDA level in blood plasma under developing pregnancy confirmed the presence of an oxidative stress. These results indicate that the variables of the antioxidative system are recommended to be analyzed in pregnant women.

In the second stage of the analysis, the women were grouped considering HPV infection (first group HPV-positive and second group HPV-negative). Antioxidative system variables were analyzed within each group but comparing data between the two trimesters of pregnancy. The results for HPV-positive and HPV-negative groups are summarized in Tables 2 and 3, respectively.

It is worth noting that GAA did not significantly change in either of the two groups, although the difference of this parameter was significant in all women tested. Moreover, the GSH / GSSG ratio significantly increased in HPV-negative women because of the nearly three times lower GSSG concentration. Although this variable was also lower in the third trimester of HPV-positive women, the difference was not so obvious, i. e., the GSH / GSSG ratio decreased in this group. This variable is known to be a very important index of the redox state of the bodily functions, implying that HPV infection depressed the antioxidative system which is crucial for the protection of pregnant women from oxidative stress.

Two variables (GSH and GSH + GSSG concentration) were found significantly decreased in cervicovaginal washing fluid while comparing the two trimesters of pregnancy in all three groups. The observation indicated that cervical tissues should be more protected from a drastical depression of the antioxidative system; also, virus infection should not contribute to this depression.

In the third stage of the experiment, HPV-positive women (n = 26) were compared with HPV-negative women (n = 40) of similar average age (30 years). The results are summarized in Table 4.

No significant difference in any variable of the antioxidative system was determined in cervicovaginal washing fluid when comparing each trimester separately. Blood plasma variables, too, showed not significant differences in the third trimester. But in the first trimester the GSSG concentration was significantly lower in HPV-infected women, although the variable was similar in two groups in the third trimester. As GSH concentration was higher in HPV-infected

Table 2. **Antioxidative system variables in blood plasma and cervicovaginal washing fluid of HPV-positive pregnant women. Variables were compared in the first (I) and the third (III) trimesters. 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 (%)**

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		

Table 3. **Antioxidative system variables in blood plasma and cervicovaginal washing fluid of HPV-negative pregnant women. Variables were compared in the first (I) and the third (III) trimesters. 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 (%)**

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		

Table 4. **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 (1) and the third (2) trimesters separately. 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**

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

women, the GSH / GSSG ratio was even twice higher in this group as well. This result allows to postulate that in an early period of pregnancy the status of glutathione pool in the antioxidative system might be rather influenced by HPV infection, while in a late period the oxidative stress seems to be the main functional and regulating factor of glutathione concentration.

Antioxidative system variables in the groups of HPV-positive and HPV-negative women were also analyzed comparing blood plasma and cervicovaginal washing fluid. No significant differences were found between the two analyzed media, although certain variables were higher in blood plasma (organism level) and others were higher in cervicovaginal washing fluid (local level).

An enhanced level of MDA was determined in the third trimester of pregnancy for all women as well as for HPV-infected and not-infected women separately. 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.

To confirm that pregnancy plays an essential role in the MDA level, the blood plasma of pregnant women (n = 115, average age 35 years) was compared with the plasma of not pregnant women (n = 58, average age 31 years). The results are summarized in Table 5.

Both in the first and the third trimesters of pregnancy, the MDA level significantly differed from that of non-pregnant women. In the early trimester, the variable for pregnant women was close to that for non-pregnant women, while with the progress of pregnancy the MDA level increased 1.6 times (Table 5). This finding confirmed that pregnancy influences the degree of lipid peroxidation. As the MDA level is recommended to be tested in the course of the fetus development, particularly in the case of restricted fetus growth, seems purposeful to test it also during normal pregnancies.

As mentioned above, the level of lipid peroxidation marker did not significantly depend on HPV infection. In contrast,

Table 5. **Malondialdehyde level in blood plasma of pregnant and not pregnant women. The variable was compared between two groups in the first (1) and third (2) trimesters of pregnancy. MDA – malondialdehyde (nmol/ml)**

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

physiological changes during pregnancy modulate the functions of the immune system – another protective system of the organism – and in the case the risk of HPV infection might be increased. These may also actuate the persistence of the virus in cervical epithelial cells and stimulate the progression of infection.

The published data concerning HPV prevalence in pregnant women are controversial. Some of them do not show any statistically significant differences in HPV prevalence the pregnant and non-pregnant women (26), while other experimental results indicate that the prevalence of HPV in pregnant women ranges from 5.5 to 65% (3). Many factors may underlie such differences, including the diversity of HPV prevalence depending on the geographical area. For example, in Austria, HPV was identified in 24.6% of 147 women studied, and HPV prevalence was lower in pregnant women older than 25 years (27). The different prevalence of HPV in pregnant (n = 164) and not pregnant (n = 153) women was found also in Turkey: the HPV infection rate was 29.2% and 19.6%, respectively (4). In a study in Japan (n = 1183), HPV was detected in 22.6% of women aged under 25 years and in 11.3% of women aged over 25 years (5). A lower rate of HPV infection (6.5%, n = 54/828) was determined in Spain (28). A high rate of HPV infection was found in Uganda. The study involved 987 primigravidae, and HPV was identified in up to 60% of pregnant women (29). In our study, the prevalence of HPV infection (17.8%) was higher than in Spain and lower than in Austria, Turkey and Japan.

Considering that infection of different HPV types exerts a specific impact on the development of cervical pathology, it is important to determine what HPV types predominate during pregnancy. Certain experimental data showed that the concentration of estrogens and progesterone increased in the blood of pregnant women, and / or the increased expression of these hormone receptors in cervix epithelial cells indirectly activated an early expression of HPV genes (30).

The published experimental data are not sufficient as concerns the importance of balance between prooxidative and antioxidative states due to physiological conditions, such as pregnancy or HPV infection. The physiological role of placental oxidative stress during pregnancy has only recently been started to be elucidated, but a number of cell functions in the placenta were recognized to be influenced by the prevailing oxygen concentration (31, 32). These functions include matrix remodeling, angiogenesis, cytotrophoblast proliferation and migration, cytotrophoblast fusion, endocrine secretion and cytokine production. Local oxidative stress in the placenta may be important for placental development, but at the same time it may exert a detrimental effect on maternal health. Alternatively, an increased maternal oxidative stress may be caused by a common insult on the placenta, characterized as an oxidative damage induced by ischemia reperfusion (31, 33). When analyzing data of the present study, we may speculate about the adaptive activation of maternal enzymatic and non-enzymatic antioxidant systems to prevent the further oxidative damage of macromolecular cell components, induced by this stress as is also suggested by other authors (34). A lower activity of the majority of antioxidative system components during the third trimester of the pregnancy was determined. According to published results, an enhanced oxidative stress in the organism of a pregnant women is typical in the early period and before delivery. The antioxidative system variables in the third trimester could be reduced in the early period of the trimester when blood plasma and cervicovaginal washing fluid were collected as there were nearly three months left to the delivery.

If there is an exaggeration of imbalance between the increased reactive oxygen / nitrogen species and defects in the maternal antioxidant defense mechanism, a systemic oxidative damage may occur and lead to pregnancy complications such as preeclampsia (31). Our data show an increased lipid peroxidation in the third trimester of pregnancy. Also, lower levels of oxidized glutathione (GSSG) and a higher GSH / GSSG ratio were determined for HPV-infected women in the first trimester of pregnancy. However, a change (drop) of GSSG concentration when comparing the two trimesters of pregnancy was more pronounced in HPV-negative women. Recently, more published data have shown that some components of the antioxidative system are also involved in inflammation processes by the mechanism of activation of stress kinases (JNK, MAPK and p38) and gene transcription factors of inflammatory mediators (NF-kappa B and AP-1) (35). Moreover, glutathione is essential for the activation of T lymphocytes and for release of cytokines as the modulation of immune response has been shown to be another important function of that antioxidant. Reduced glutathione concentration in blood and tissues was due to virus infections such as influenza, hepatites B and C or dengue fever (36). Here, it could be premised that the concentration

of oxidized glutathione reduced by half in the third trimester of pregnancy in HPV-positive women and three times in non-infected women due to enhanced requirement of functional (reduced) glutathione to provide the anti-inflammatory effect.

Increased MDA levels were determined in cases of human cervical carcinoma (37). No significant differences of the variable were determined in our study for pregnant women due to HPV infection, although the MDA level tended to be higher in HPV-infected women. This result may indicate that this variable could not be considered as an additional biomarker of cervical carcinoma risk.

To sum up, oxidative unbalance during pregnancy, especially in pathological states (pregnancy-induced hypertension, preeclampsia and gestational diabetes), might be even deepened by changes of antioxidative system variables as determined in the present research. On the other hand, persistence of HPV infection is known also to be regulated by the level of oxidative stress. All those facts should be considered if premising the importance of additional screening for HPV-infected women after delivery.

CONCLUSIONS

1. A decrease of GSH concentration and an increase of MDA level in blood plasma under development of pregnancy confirmed the presence of a general oxidative stress. Lower GSH and GSH + GSSG concentrations in the cervicovaginal washing fluid while comparing two trimesters of pregnancy were markers of a local oxidative stress.

2. In the first trimester of pregnancy, the GSSG concentration in blood plasma was significantly lower and the GSH / GSSG ratio was even twice higher in HPV-infected women. While comparing HPV-positive and HPV-negative women, no significant difference on any variable of the antioxidative system was determined in the cervicovaginal washing fluid. Thus, HPV infection was found to depress the antioxidative system at the body level and not to affect this protective system at the local level. Moreover, the status of the glutathione pool might be rather influenced by HPV infection at an early period of pregnancy, while oxidative stress seems to be the main factor affecting and regulating the glutathione level in the late period of the pregnancy.

3. Enhanced levels of MDA in the third trimester of pregnancy were determined in all women, but only slightly higher values of this variable were determined due to HPV infection. The result might indicate that systemic oxidative stress during pregnancy affects lipid peroxidation as a more sensitive process, while it does not markedly depend on HPV infection. As the level of MDA is recommended to be tested in the course of the fetus development, particularly in cases of restricted fetus growth, the parameter could be suggested also to be tested during a normal pregnancy, but this variable should not be considered as an additional biomarker of cervical carcinoma risk.

4. Oxidative imbalance during pregnancy, especially in a pathological state, might be deepened by changes of antioxidative system variables, and the persistence of HPV infection depends on the level of oxidative stress. Additional screening for HPV-infected women after delivery is recommended.

Received 10 October 2010
Acctepted 7 December 2010

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Birutė Surinėnaitė, Gintautas Domža, Janina Didžiapetrienė, Gražina Prasmickienė, Živilė Gudlevičienė, Audronė Jakaitienė

ŽPV INFEKUOTŲ NĖŠČIŲJŲ ANTIOKSIDACINĖS SISTEMOS BŪKLĖ

Santrauka

Tikslas. Tyrimo tikslas buvo nustatyti redukuoto (GSH), oksiduoto (GSSG) ir bendro (GSH + GSSG) glutationo koncentracijos, malono dialdehido (MDA) kiekio, taip pat bendrojo antioksidacinio aktyvumo (BAA) rodiklius nėščių moterų kraujo plazmoje ir gimdos kaklelio nuoplovų bandiniuose, esant ir nesant žmogaus papilomos viruso (ŽPV) infekcijai.

Tiriamieji ir metodai. Tiriamųjų grupę sudarė 213 nėščių moterų, 2008–2010 m. užsiregistravusių Centro poliklinikos diagnostikos centre. Šios moterys ištirtos dėl ŽPV infekcijos, o ją aptikus – dėl viruso tipo. Testai atlikti pirmame ir trečiame nėštumo trimestruose surinkus kraujo plazmos ir gimdos kaklelio nuoplovų bandinius. MDA kiekis nustatytas tiobarbitūrinės rūgšties (TBR) testu, glutationo koncentracija – dviguba reakcija su 5,5'-ditiobis-2-nitrobenzoine rūgštimi (DTNB) ir glutationo reduktaze (GR), atitinkamai paruošus bandinius be ar su 2-vinilpiridinu. BAA nustatytas taikant Tween 80 oksidacijos testą. Visi rodikliai išsiaiškinti spektrofotometriniu metodu.

Rezultatai. Visų tirtų nėščiųjų kraujo plazmoje nėštumo metu buvo sumažėjusi abiejų glutationo formų koncentracija, taip pat BAA ir padidėjęs MDA kiekis. Gimdos kaklelio nuoplovose pasikeitė tik du iš minėtų rodiklių. Lyginant ŽPV infekuotas ir neinfekuotas nėščias moteris, BAA pokyčių nerasta. GSH / GSSG santykis

padidėjo ŽPV neturinčioms ir sumažėjo šio viruso nešiotojoms. Lyginant panašaus amžiaus grupių infekuotas ir neinfekuotas moteris trečiame nėštumo trimestre, gimdos kaklelio nuoplovose ir kraujo plazmoje nepastebėta nė vieno iš tirtų rodiklių reikšmingo pokyčio, tuo tarpu pirmame trimestre infekuotųjų ŽPV grupėje nustatyta žemesnė GSSG ir didesnė GSH koncentracija, taip pat didesnis GSH / GSSG santykis. Siekiant patvirtinti nėštumo įtaką lipidų peroksidacijai ir jo rodiklio – MDA – kiekiui, visų nėščiųjų kraujo plazma palyginta su panašaus amžiaus nenėščių moterų kraujo plazma. Pirmame nėštumo trimestre šis rodiklis moterų kraujyje buvo artimas nenėščių moterų kraujyje nustatytam MDA kiekiui, tačiau nėštumo metu jis padidėjo 1,6 karto. Šio rodiklio skirtumų priklausomybė nuo ŽPV užkrato nėščiosioms nenustatyta.

Išvados. GSH koncentracijos sumažėjimas ir MDA kiekio padidėjimas kraujo plazmoje nėštumo metu patvirtina oksidacinio streso išsivystymą organizme. Mažesnė GSH ir GSH + GSSG koncentracija gimdos kaklelio nuoplovose, lyginant du nėštumo trimestrus, gali rodyti vietinio lygmens oksidacinį stresą. ŽPV infekcija slopina antioksidacinės sistemos funkciją nėščiųjų organizme ir praktiškai neveikia šios sistemos rodiklių vietiškai. Lipidų peroksidacijai nėštumo metu turi įtakos ne tiek ŽPV užkratas, kiek sisteminis oksidacinis stresas. MDA kiekį rekomenduojama sekti ne tik esant nėštumo patologijai, bet ir normaliai jo eigai, nors remiantis šio tyrimo duomenimis, minėtas rodiklis neturėtų būti vertinamas kaip papildomas gimdos kaklelio karcinomos riziką rodantis žymuo. Antioksidacinės sistemos rodiklių pokyčiai gali pagilinti oksidacinį disbalansą vystantis patologiniam nėštumui, tuo tarpu oksidacinis stresas gali būti viena iš ŽPV infekcijos pasireiškimo priežasčių, todėl svarbu ŽPV užsikrėtusias nėščiąsias periodiškai tirti ir po gimdymo.

Raktažodžiai: ŽPV infekcija, nėštumas, antioksidacinė sistema