ACTA MEDICA LITUANICA. 2008. Vol. 15. No. 3. P. 140–144

Host response to *Human papilloma virus* in cervical cancer patients

Birutė Kazbarienė,

Gražina Prasmickienė,

Aurelija Krikštaponienė,

Živilė Gudlevičienė,

Janina Didžiapetrienė

Laboratory of Cancerogenesis and Tumor Pathophysiology, Institute of Oncology, Vilnius University, Lithuania

Background. Disbalance of immune and antioxydative systems are among the risk factors activating *Human papilloma virus* (HPV) infection and development of cervical cancer. The aim of this study was to investigate functions of these systems in cervical cancer patients analyzing alterations related to the types of HPV infection.

Methods. 136 cervical cancer patients were tested for HPV infection. Parameters of cellular and humoral immunity were analyzed. The antioxydative system status was evaluated detecting the level of lipid peroxydation product malondialdehyde (MDA), the catalytic activity of antioxidant enzymes catalase (CAT) and superoxyde dismutase (SOD) and the amount of antioxidant vitamins A and E.

Results. In cervical cancer patients group infected with HPV types 16 or 18 five indices of immunity ($CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$, IgM) were lower comparing with the corresponding parameters of HPV-negative patients. In HPV-positive (unidentified types) patients $CD16^+$ and $CD19^+$ were lower than in HPV-negative patients. In patients infected with HPV types 16 or 18 $CD4^+$ and $CD16^+$ were found to be lower if compared with the corresponding indices of HPV-positive (unidentified types) patients. No significant changes of the antioxydative system parameters were observed in patients infected by HPV types 16 or 18 comparing to the patients infected by HPV of unidentified types or to HPV-negative patients.

Conclusions. Alterations of immune system parameters of cervical cancer patients were determined by HPV infection. Antioxydative system parameters were found not to be changed significantly due to the infection.

Key words: cervical cancer, HPV, antioxydative system, immune system

INTRODUCTION

Human papilloma virus (HPV) infection is the crucial risk factor in the development of cervical cancer (1). However, mechanisms of HPV-induced cervical cancerogenesis are still not definitely clear. Moreover, factors determining spontaneous disappearance or persistence of HPV infection have been not identified. Apparently, the status of immune and antioxydative systems of the organism can play a certain role in the processes mentioned. Results of the previous studies (2–4) showed functions of cellular and humoral immunity to be affected by HPV infection. Some authors also suggested there were significant alteration of the antioxydative system status of cervical cancer patients under HPV infection (5, 6). In our previous report, where parameters indicating the status of the antioxydative system in blood serum of cervical cancer patients infected and uninfected by HPV were analyzed, no statistically significant difference was determined (7). Thereby, the data on this point are contrasting. On the other hand, proteins of oncogenic HPV type E6 and E7 were shown to destroy the regulatory mechanism of a cell cycle inhibiting *p53* and retinoblastoma (*Rb*) gene functions and directing to an uncontrolled cell division. Under disturbance of *p53* that induces DNA reparation and apoptosis and protects a genome from free radical-induced DNA damages (8) the catalytic activity of the antioxydative enzymes can be reduced while function of *p53* and the enzymatic pool of the antioxydative system are in balance under normal conditions (9). The role of both systems of the organism protection mentioned in cervical cancerogenesis is not fully understood, consequently, further investigations into the subject are necessary. The aim of the present research was to investigate if HPV infection affects the status of immune and antioxydative systems of cervical cancer patients in relation to oncogenicity of HPV infection as well.

MATERIALS AND METHODS

Patients. 136 cervical cancer patients (aged 21–78 years) were involved in the study. 98 women were diagnosed with squamous cell carcinoma (SCC), 11 women suffered from cervical adenocarcinoma (AD) and 27 women were found to carry carcinoma *in situ*.

HPV detection by PCR. Polymerase chain reaction (PCR) with general primers MY09/MY11 and type specific primers for HPV 16 and 18 (TS-PCR) were used for the detection and typing

Correspondence to: Birutė Kazbarienė, Institute of Oncology, Vilnius University, Baublio 3B, LT-08660 Vilnius, Lithuania.
E-mail: birute.kazbariene@loc.lt

of HPV. Other HPV types were detected by sequencing. Prior to testing all the samples were stored at –20 °C.

Immune system parameters. Parameters of cellular immunity were determined for all the women by indirect immunofluorescence method using monoclonal antibodies (Dako A\S, Denmark). The percentage and absolute number of T lymphocyte population ($CD3^+$), T helpers / inductors ($CD4^+$), T supressors / cytotoxic lymphocytes ($CD8^+$), immunoregulation index ($CD4^+/CD8^+$), B lymphocyte ($CD19^+$), natural killer (NK) cells ($CD16^+$) were detected. To evaluate humoral immunity, IgG, IgA and IgM concentration in blood serum were examined (10). The haematological parameters were determined by haematological analyzer (Coulter HmX, Beckman).

Antioxydative system parameters. Antioxydative system status was evaluated by measuring the level of a lipid peroxidation product malondialdehyde (MDA), the activity of the antioxydative enzymes catalase (CAT) and superoxide dismutase (SOD) and the amount of endogenous antioxidant vitamins A and E. The level of MDA was determined by the thiobarbituric acid (TBA) assay (Sigma, Germany) (11). CAT activity was determined as the formation of a colour complex of hydrogen peroxide and ammonium molybdate. SOD activity was detected as the rate of inhibition of nitro blue tetrazolium reduction (Sigma, Germany) in erythrocyte haemolysate. All the parameters were measured spectrophotometrically. Serum levels of vitamins A and E were estimated in hexane extract by fluorimetric method.

Statistical analysis. Statistical analysis was made using the statistical software SAS version 8.2. The differences of parameters were estimated as reliable when $p \leq 0.05$ and in tendency when $0.05 < p \leq 0.1$.

RESULTS

HPV type prevalence in cervical cancer patients. In cancer patients group high-risk HPV types (HPV 16, 18) were detected for 86 women, including HPV 16 for 80 women, HPV 18 for 6 women. 25 patients were infected with HPV, but types of virus were not identified, and 25 patients were HPV-negative.

Status of immune and antioxydative systems under HPV infection. Comparing parameters indicating the immune system status of cervical cancer patients infected with HPV and of those not-infected, the percentage of $CD19^+$ lymphocytes was significantly lower and the concentration of IgM in the serum had a tendency to be lower for the infected patients.

Evaluating the influence of HPV infection on the antioxydative system parameters in serum of cervical cancer patients, concentration of the lipid peroxydation product MDA, the activity of antioxydative enzymes CAT and SOD and the amount of endogenous antioxidant vitamins A and E were compared in the HPV infected and not-infected patients groups. No statistically significant differences between corresponding antioxydative system parameters were found considering HPV infection of cervical cancer patients.

Table 1. **Comparison of the immune system parameters of cervical cancer patients (HPV-negative, HPV-positive types 16, 18 or unidentified types)**

Groups	n	Leukocytes	Lymphocytes		Monocytes		Neutrophils	
		$n \cdot 10^9/L$	%	$n \cdot 10^9/L$	%	$n \cdot 10^9/L$	%	$n \cdot 10^9/L$
Patients (HPV-negative)	25	6.6 ± 0.28	29 ± 1.9	1.9 ± 0.13	8 ± 0.5	0.5 ± 0.03	59 ± 1.9	3.9 ± 0.22
Patients (HPV-positive, types 16, 18)	86	6.3 ± 0.23	30 ± 0.9	1.8 ± 0.06	8 ± 0.3	0.5 ± 0.02	60 ± 0.9	3.9 ± 0.18
Patients (HPV-positive, unidentified types)	25	6.9 ± 0.41	28 ± 1.6	1.9 ± 0.10	8 ± 0.6	0.5 ± 0.03	61 ± 2.1	4.4 ± 0.40

Groups	n	$CD3^+$		$CD4^+$		$CD8^+$		$CD4^+/CD8^+$	B($CD19^+$)		NK($CD16^+$)	
		%	$n \cdot 10^9/L$	%	$n \cdot 10^9/L$	%	$n \cdot 10^9/L$		%	$n \cdot 10^9/L$	%	$n \cdot 10^9/L$
Patients (HPV-negative)	24	62 ± 0.7	1.2 ± 0.09	41 ± 0.5	0.8 ± 0.05**	34 ± 0.3	0.7 ± 0.05**	1.2 ± 0.02	*11 ± 0.4**	0.2 ± 0.02	*19 ± 0.3	*0.4 ± 0.03
Patients (HPV-positive, types 16, 18)	76	62 ± 0.4	1.1 ± 0.04	41 ± 0.3	*0.7 ± 0.02**	34 ± 0.2	0.6 ± 0.02**	1.2 ± 0.01	*10 ± 0.2	0.2 ± 0.01	*18 ± 0.2	*0.3 ± 0.01
Patients (HPV-positive, unidentified types)	25	62 ± 0.6	1.1 ± 0.07	41 ± 0.4	*0.8 ± 0.04	34 ± 0.3	0.6 ± 0.04	1.2 ± 0.02	10 ± 0.4**	0.2 ± 0.01	*19 ± 0.4	*0.3 ± 0.02

Groups	n	Ig(g/L)		
		IgG	IgA	IgM
Patients (HPV-negative)	14	15.1 ± 1.06	2.3 ± 0.34	1.2 ± 0.11
Patients (HPV-positive, types 16, 18)	66	14.6 ± 0.56	2.3 ± 0.14	1.0 ± 0.04
Patients (HPV-positive, unidentified types)	24	14.8 ± 0.99	2.6 ± 0.25	1.0 ± 0.06

* $p < 0.05$.

** $0.05 \leq p < 0.1$.

Note. Different symbols designate the compared groups.

Table 2. **Antioxydative system parameters of cervical cancer patients (HPV-negative, HPV-positive, type 16, 18 or unidentified types)**

Parameters	Patients		
	HPV-negative n = 25	HPV 16, 18 types n = 86	Unidentified types n = 25
MDA, nmol/ml	14.7 ± 0.5	15.3 ± 0.5	15.3 ± 0.7
CAT, nmol/l/min	29.9 ± 1.8	29.6 ± 1.5	31.8 ± 1.9
SOD, U/ml•10^4	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Vit. E, µmol/l	25.9 ± 1.4	25.6 ± 1.0	28.1 ± 1.3
Vit. A, µmol/l	2.9 ± 0.1	3.2 ± 0.1	3.2 ± 0.1

Immune and antioxydative systems parameters of cervical cancer patients subject to HPV infection types. Comparison of all the parameters analyzed in the group of the cervical cancer patients infected by HPV 16 or 18 types with the corresponding data of HPV-negative patients group showed that the percentage of $CD19^+$, percentage and absolute number of $CD16^+$ indices were significantly lower, absolute number of $CD4^+$ and $CD8^+$ were in tendency lower for HPV-infected patients. The absolute number of $CD4^+$ and percentage of $CD16^+$ were reliably lower for patients infected by high-risk HPV types than for HPV-positive patients of unidentified types of the infection. The percentage of $CD19^+$ and absolute number of $CD16^+$ were lower in tendency for HPV-positive patients of unidentified virus type if compared with the corresponding parameters of HPV- negative patients (Table 1).

No significant differences of any antioxydative system parameters analyzed (MDA concentration, CAT and SOD activities, and vitamins A and E levels) were detected for cervical cancer patients infected by HPV of high-risk types 16 or 18 compared to the patients infected by HPV of unidentified types (Table 2).

DISCUSSION

The cellular immunity is one of the crucial factors in controlling the HPV infection. Insufficient activation of cellular cytotoxic mechanisms can determine persistence of the infection (12).

Our previous data showed that certain parameters of cellular immunity (lymphocytes, $CD3^+$, $CD4^+/CD8^+$, $CD19^+$, $CD16^+$) were lower for the women with cervical cancer or precancerous lesions comparing with the parameters in healthy women group (3). The results were supported by other authors (13). However, our data indicated certain other parameters (monocytes, neutrophils and its phagocytes activity, $CD8^+$, concentrations of IgG, IgA, IgM in serum) to be at higher level. Moreover, the immune system parameters for cervical cancer patients were found to be under the influence of the disease stage. The weakening of the immunity was also revealed under progression of the malignancy process (4).

As HPV of 16 or 18 types is known as certain biological carcinogens, consequently, comparison of immune system parameters of patients infected by viruses of these types with both HPV-negative and HPV-positive (of unidentified types) patients group was provided. The present study showed that decrease of particular immune system parameters ($CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$) is typical to patients infected with HPV 16 or 18 types but not to HPV-negative cervical cancer patients while only two parameters ($CD19^+$ and $CD16^+$) were lowered for patients infected with HPV of unidentified types. Therewith two parameters ($CD4^+$ and $CD16^+$) were lowered for patients infected with HPV 16 or 18 types comparing with HPV-positive patients with unidentified type of HPV infection. Thus, it is evident that most parameters of cellular immunity were reduced for patients infected with HPV 16 or 18 types. According to other authors regulatory T cells ($CD4^+$/CD25 (high) T cell in $CD4^+$ T cell fraction) demonstrate increased frequencies and suppressive activity, therefore, they may inhibit immune response and control of cervical neoplasia (14). It was also shown that the HPV 16 specific $CD4^+$ T cell response in cervical cancer patients is either absolutely neutralized or strongly impaired (15). It should be emphasized that in patients group infected with HPV 16 or 18 and unidentified types the number of natural killer ($CD16^+$) cells was also lower (16). The cells are known to be most important for tumour cells elimination and antiviral protection of the organism.

Dysfunction of the immune system can be an endogenic risk factor activating HPV infection and promoting the development of cervical cancer. It was shown that deficiency of immune functions can also promote the malignant process (17).

In line with our previous results, MDA concentration increased nearly 1.5-fold while the activity of antioxydative enzyme SOD decreased 1.6-fold to cervical cancer patients compared to the same parameters in the serum of control women group. CAT activity was higher in the cancer patients group (3). Few other reports were in agreement with the results (18–20). Here we showed the level of endogenous antioxidant, vitamins A and E in the serum of cancer patients not to be significantly altered if compared to the control group.

To resume, in the case of cervical cancer the equilibrium between the prooxydant and antioxidant processes in the organism is disturbed. Dominating prooxydative state results in oxydative stress leading to a significantly reduced capacity of the antioxydative system.

Considering the depressed capacity of antioxydative system in the case of cervical cancer it was important to study the dynamics of the changes of the antioxydative system status depending on HPV infection. The levels of vitamins A and E in serum of HPV-positive cancer patients were found to be lower than those of HPV-negative cancer patients (5, 6). Analyzing the influence of HPV infection on the status of antioxydative system, no statistically significant differences of any parameters were determined if comparing HPV infected and not-infected women. Also no significant variation of any antioxydative system parameters were found to compare the patients infected by HPV of 16 or 18 types to those infected by other types of HPV.

It could be suggested that the increased lipid peroxydation and overall reduced capacity of the antioxydative system for cervical cancer patients is related to the cancer process rather than to HPV infection. The relation of the antioxydative system status with the stage of the disease was analysed in our previous work (4).

According to the data, capacity of the antioxydative system of cervical cancer patients was impaired although the relation of the system status and the stage of the disease was found to be ambiguous. Catalytic activity of SOD and the concentration of vitamin E were assumed to be upregulated in the advanced stages of cervical cancer in comparison to earlier stages.

CONCLUSIONS

Most of the immune system parameters investigated in our study were found to be lower in cervical cancer patients infected with HPV types 16 or 18, while the antioxydative system parameters of cervical cancer patients were determined not to change significantly due to HPV infection.

Received 27 May 2008
Accepted 23 September 2008

References

1. Karube A, Sasaki M, Tanaka H, Nakagome O, Dahiya R, Fujimoto S and Tanaka T. Human papillomavirus type 16 infection and the early onset of cervical cancer. Biochem Biophys Res Commun 2004; 323: 621–4.
2. Nakagawa M, Viscidi R, Deshmukh I, Costa MD, Palefsky JH, Farhat S, Moscicky AB. Time course of humoral and cell-mediated immune responses to *Human papilloma virus* type16 in infected women. Clin Diagn Lab Immunol 2002; 9(4): 877–82.
3. Kazbariene B, Prasmickiene G, Krikstaponiene A, Sukeliene D, Burneckis A, Didziapetriene J. Changes in the parameters of immune and antioxidant systems in patients with cervical cancer. Medicina 2004; 40: 1158–63,
4. Didžiapetrienė J, Kazbarienė B, Prasmickienė G, Krikštaponienė A, Surinėnaitė B. The immune and antioxidative state of women with respect to cervical cancer stage. Acta Medica Lituanica 2005; 12(3): 65–70.
5. Kwasniewska A, Tukendorf A. Level of retinol in blood serum of women infected with HPV and with dysplastic changes of the cervix. Med Dosw Mikrobiol 1996; 48: 71–7.
6. Kwasniewska A, Tukendorf A, Semczuk M. Content of alpha-tocopherol in blood serum of *Human papilloma virus*-infected women with cervical dysplasias. Nutr Cancer 1997; 28: 248–51.
7. Didžiapetrienė J, Prasmickienė G, Šukelienė D, Gudlevičiene Z, Žemaitytė R, Jurgelevičiene V. Antioksidacinės sistemos būsena, sergant gimdos kaklelio vėžiu ir esant žmogaus papilomos viruso infekcijai. Sveikatos mokslai 2003; 13(7): 44–7.
8. Das UN. A radical approach to cancer. Med Sci Monit 2002; 8: 79–92.
9. Pani G, Bedogni B, Anzevino R, Colavitti R, Palazzotti B, Borrello S, Galeotti T. Deregulated manganese superoxide dismutase expression on resistance to oxidative injury in *p*53-deficient cells. Cancer Res 2000; 60(16): 4054–60.
10. Mancini C, Carbonara A, Heremens J. Immunochemical quantization of antigen by single radial diffusion. Immunochem 1965; 20: 235–48.
11. Surinėnaitė B, Kazbarienė B, Prasmickienė G, Krikštaponienė A, Didžiapetrienė J. Surgical stress induced alterations of antioxidative and immune system parameters. Biology 2006; 2: 76–9.
12. Lee BN, Follen M, Shen DY, Malpica A, Adler-Storthz K, Shearer WT, Reuben JM. Depressed type 1 cytokine synthesis by superantigen-activated $CD4^+$ T cells of women with *Human papilloma virus*-related high-grade squamous intraepithelial lesions. Clin Diagn Lab Immunol 2004; 11: 239–44.
13. Lazarenko L, Spivak M, Lakatosh V, Kryvokhadska L, Mikhailenko O, Rudenko A, Tkacikova L, Mikula I. Production of interferon and change of the lymphocyte subpopulation phenotype in peripheral blood at cervical *papilloma virus* infection. Folia Microbiol 2002; 47: 747–52.
14. Visser J, Nijman HW, Hoogenboom BN, Jager P, van Baarle D, Schuuring E, Abdulahad W et al. Frequencies and role of regulatory T cells in patients with (pre)malignant cervical neoplasia. Clin Exp Immunol 2007; 150(2): 199–209.
15. de Jong A, van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, Kenter G et al. *Human papilloma virus* type 16-positive cervical cancer is associated with impaired $CD4^+$ T-cell immunity against early antigens E2 and E6. Cancer Res 2004; 64(15): 5449–55.
16. Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, Coccol L, Vitale M. NK cells and cancer. J Immunol 2007; 178(7): 4011–16.
17. Hadden JW. Immunodeficiency and cancer: prospects for correction. Int Immunopharmacol 2003; 3: 1061–71.
18. Ahmed MI, Fayed ST, Hossein H and Tash FM. Lipid peroxydation and antioxydant status in human cervical carcinoma. Dis Markers 1999; 15: 283–91.
19. Manju V, Kalaivani Sailaja J, Nalini N. Circulating lipid peroxydation and antioxydant status in cervical cancer patients: a case-control study. Clin Biochem 2002; 35: 621–5.
20. Lee GJ, Chung HW, Lee KH Ahn HS. Antioxidant vitamins and lipid peroxydation in patients with cervical intraepithelial neoplasia. J Koream Med Sci 2005; 20: 267–72.

Birutė Kazbarienė, Gražina Prasmickienė, Aurelija Krikštaponienė, Živilė Gudlevičienė, Janina Didžiapetrienė

ORGANIZMO ATSAKAS Į ŽPV INFEKCIJĄ SERGANT GIMDOS KAKLELIO VĖŽIU

S a n t r a u k a

Tikslas – ištirti gimdos kaklelio vėžiu sergančių moterų imuninės ir antioksidacinės sistemų parametrus priklausomai nuo žmogaus papilomos viruso (ŽPV) tipų.

Pacientai ir metodai. Tirtos 136 pacientės. ŽPV 16 tipas buvo nustatytas 80 moterų, ŽPV 18 – 6 moterims. Kitais (neidentifikuotais) ŽPV tipais buvo infekuotos 25 tiriamosios. Nebuvo užsikrėtusios 25 pacientės.

Tiriamosioms nustatyti ląstelinio ir humoralinio imuniteto rodikliai ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$, $CD4^+/CD8^+$, IgG, IgA, IgM). Sergančiųjų antioksidacinė būklė vertinta pagal lipidų peroksidacijos produkto malono dialdehido (MDA) koncentraciją, antioksidacinių fermentų katalazės (KAT) ir superoksiddismutazės (SOD) aktyvumą bei endogeninių antioksidantų vitaminų A ir E kiekį kraujo serume.

Rezultatai. Gimdos kaklelio vėžiu sergančių ir infekuotų ŽPV 16 ar 18 tipais moterų imuninės sistemos rodiklių ($CD4^+$, $CD8^+$, $CD19^+$, $CD16^+$) reikšmės buvo mažesnės, palyginus su ŽPV neinfekuotų sergančiųjų rodiklių reikšmėmis. Pacienčių, infekuotų kitais ŽPV tipais, tik $CD19^+$ procentinis rodiklis ir absoliutus $CD16^+$ kiekis buvo mažesni už atitinkamus rodiklius ŽPV neinfekuotų pacienčių grupėje. Infekuotų ŽPV 16 ir 18 tipais grupėje $CD4^+$ rodiklio absoliuti reikšmė ir procentinis $CD16^+$ rodiklis buvo patikimai mažesni, palyginus su šių rodiklių reikšmėmis sergančiųjų grupėje, infekuotų kitais ŽPV tipais.

Tiriant ŽPV įtaką antioksidacinės sistemos būklei statistiškai reikšmingo skirtumo tarp ŽPV infekuotų ir neinfekuotų tiriamųjų nenustatyta. Tarp pacienčių, infekuotų didelės onkogeninės rizikos 16 ar 18 tipų ŽPV ir infekuotų kitais ŽPV tipais, patikimo skirtumo taip pat nenustatyta.

Išvados. Moterų, sergančių gimdos kaklelio vėžiu, imuninės sistemos parametrų pokyčiai priklauso nuo ŽPV infekcijos, tuo tarpu antioksidacinės sistemos pokyčiai nuo ŽPV infekcijos tipų nepriklauso.

Raktažodžiai: gimdos kaklelio vėžys, ŽPV, antioksidacinė sistema, imuninė sistema