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PERIPHERAL BLOOD LYMPHOCYTES PHENOTYPIC COMPOSITION IN PATIENTS WITH CERVICAL PAPILOMAVIRUS INFECTIONS, WHICH IgG ANTIBODY TO HERPES SIMPLE VIRUSES WERE DETERMINED

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The aim of the work – to determine the phenotypic composition of peripheral blood lymphocytes in patients with cervical papillomavirus infection (PVI), whose blood serum showed IgG antibodies to herpes simplex viruses with (HSV)-1 and/or (HSV)-2.

Materials and methods. 43 women (average age 26.5 years old) with human papillomavirus (HPV)-induced cervical papillomavirus infection diseases diagnosed by molecular-biological, colposcop, and cytological methods of examination were inspected. Serum screening for anti-HSV-1 and anti-HSV-2 IgG as well as examination of anti-HSV-2 IgG avidity index were examined by solid-phase enzyme-linked immunosorbent assay. The phenotypic composition of peripheral blood lymphocytes were examined by the flow cytofluorometry method using monoclonal antibodies (Beckman Coulter, USA) to CD3⁺, CD4⁺, CD8⁺, CD3⁺/HLA-DR⁺, CD3⁺/CD16⁺/CD56⁺, CD4⁺45RA⁺ CD16⁺/CD56⁺/CD8⁺ and CD16⁺/CD56⁺ antigens. The count of cells and data analysis were performed by cytofluorometer FC-500 (Beckman Coulter, США). The count of cells and analysis of the results were performed by cytofluorometer i. IgG antibodies to HSV-1, HSV-2 or to these two viruses in serum of patients with cervical PVI were detected in 90.0 % of cases, in which the clinical symptoms of herpetic infection were no identified. IgG antibodies to HSV-2 in serum of patients had only mean or low avidity. In patients with IgG antibodies to HSV-2 or simultaneously with HSV-2 and HSV-1, and, above all, with low-avidity IgG antibodies to HSV-2 the frequency of HPV-induced cervical intraepithelial neoplasia (CIN) of I-II and III grade and cancer in situ was increased. The number of CD3⁺, CD4⁺, CD8⁺, CD4⁺45RA⁺ and CD16⁺/CD56⁺ cells in the patients' peripheral blood, as well as the CD4⁺/CD8⁺ index were not altered compared to the indicators for clinically healthy subjects (control). But the tendency to increase the number of CD16⁺/CD56⁺/CD8⁺ cells, as well as a significant increase in the number of CD3⁺/HLA-DR⁺

cells were determined. The number of CD3⁺/CD16⁺/CD56⁺ cells was reduced in peripheral blood of patients with low-avidity of IgG antibodies to HSV-2, whereas in the remaining patients it was at the control level. meter FC-500 (Beckman Coulter, USA).

Results and discussion. In patients serum with CU HPV I without clinical signs of herpetic infection to HSV-1, HSV-2 IgG antibodies, both these two viruses simultaneously were diagnosed in 90.0 % of cases. Anti-HSV-2 IgG in patients' serum had only medium or low avidity. The frequency of revealing HPV-induced cervical intraepithelial neoplasia (CIN) I-II and III degree of activity, and cancer in situ increased in patients with anti-HSV-2 IgG or simultaneously to anti-HSV-1 and anti-HSV-2 but, first of all, in patients with low avidity anti-HSV-2 IgG. The number of CD3⁺, CD4⁺, CD8⁺, CD4⁺45RA⁺ and CD16⁺/CD56⁺ cells in the patients' peripheral blood, as well as CD4⁺/CD8⁺ index did not change as compared with group of clinically healthy persons (control group). However, there was determined the tendency of CD16⁺/CD56⁺/CD8⁺ cells number increase and significant CD3⁺/HLA-DR⁺ cells number increase. CD3⁺/CD16⁺/CD56⁺ cells number in peripheral patients' blood with low avidity to anti-HSV-2 IgG antibodies was reduced whereas it was equal to control level in the rest patients.

Conclusion. In patients with CU HPV I without clinical manifestation of herpetic infection anti-HSV-2 low avidity IgG were identified. The frequency of more severe CIN cause was increased. There were more significant displacement of cell immunity confirmed by CD3⁺/HLA-DR⁺ cells number increase and tendency of CD16⁺/56⁺CD8⁺ cells number increase, too, on the background of CD3⁺/CD16⁺/CD56⁺ cytotoxic T-lymphocytes decrease. Complex individual treatment of these patients with anti-herpetic drugs must probably be used.

Key words: papillomaviruses; herpesviruses; cervix; lymphocytes; natural killer; peripheral blood.

Despite successes of the development of complex person-oriented (individual) treatment of patients with papillomavirus infection (HPVI) and introduction of human papillomavirus vaccine into the health care during the last decades in all countries of the world, there is tendency of increase of human papillomavirus induced (HPV) pre-tumor diseases of anogenital region including cervix uteri (CU), which takes second place among women tumor pathology. HPVI of anogenital region in women is observed in 2–44.0 % of cases. This disease has asymptomatic course and spontaneous recovery is observed in 90 % of cases. In 10–15 % of cases there is a lifelong persistence of HPV, that's why sometimes it may lead to disease progression and eventually different invasive cancer forms development. HPV DNA of cervical carcinoma of high risk is examined in 99.0 % of cases. The most spread HPV are types 16 and 18 which may be found in about 70.0 % of cases.

For enhancement of diagnosis, prevention and treatment of patients with PVI, special biomarkers panel for detection of HPV-induced pre-tumor and tumor diseases of anogenital region such as phenotypic (proliferation and differentiation, hystological, dysplastic, and viral receptors to hormones, and growth factors, tumor antigens etc.), genotypic (HPV oncogenic types, p53, bcl-2, bax etc. genes activity), immunological (cytokines production changes, suppression of γ -interferon production, CD4⁺ cells number reduction, Th1/Th2 immune response imbalance etc.), visual (colposcopy, ultrasound and immunohistochemical examinations etc.), and anamnestic data [4]. This panel is constantly studied and extended to stop disease and mortality ratio from cervix uteri cancer.

It is known that HPV persistence and its carcinogenic effect are closely related both with the processes of integrating of HPV DNA with high oncogenic risk into the host cell genome and risk factors of HPV and oncogenic transformation of HPV-infected cells [5]. Among them there are microenvironment changes near HPV-infected epithelium of cervix uterus [6], genetic and epigenetic factors (MHC, genes polymorphism, p53 proteins), action of unfavorable conditions of environment (ultrasound radiation, chemical agents, stress) [7], immune system infringements [8], and patients' infection by other sexual transmitted pathogens (Epstein-Barr virus, Herpes simplex virus. HIV, Chlamydia etc.) [9].

Factors of innate and acquired immune responses are largely effective in stopping primary HPV infections in most cases, and they have an important role in controlling the course of HPV, and the growth of HPV-induced tumors [1, 4, 10]. During evolutionary development, HPV received ability to resist the immune system, and even cause immunosuppression in the mucous membrane of the epithelium of cervix of uteri. It is known today, that the main

mechanisms of HPV immunosuppressive activity except the absence of viremia stage; slow synthesis of capsid proteins which have high immunogenicity, and low expression of its oncoproteins on the surfaces of infected cells, and suppression of some antigenprocessing stages; increase of immunosuppressive cytokines production, which inhibit Th-1 development, and γ -interferon (IFN γ) and interleukin-2 (IL-2); decrease the *Langengarce's* cells number or full disappearance in HPV-infected epithelial cells; TLR- expression changes etc. [4, 10, 11]. It was established that the ability of HPV to avoid antiviral immunity factors and first of all, its cellular line leads to the development of chronic inflammation and finally, to the appearance and progression of HPV-induced cancer [12].

Infection of patients with HSV-1 and HSV-2 is important cofactor of HPV CU. Cofactor action of Herpesviruses in HPV-induced tumor diseases is connected not only with their influence on HPV oncoproteins E6 and τ E7 [13] but inflammation [14] and immune suppression development [15]. Genital herpes is one of the most wide-spread common disease with sexual way of transmission, and differs from other disease of this group by its life carriage. This effect is responsible for great percent of recidivation. Herpetic infection can have atypical, subclinical, and asymptomatic cause of diseases. It is frequent reason of diagnostic mistakes and untimely diagnosis of the illness. Therefore, in complex laboratory diagnosis of herpetic infection serologic methods must be used additionally for identification, first of examination of virus-specific IgG as detection of anti-HSV IgM has a limited value for early confirmation of acute infection. Examination of the avidity index of anti-HSV IgG is also recommended but experience of this method use in routine laboratories is limited too [16].

Abovementioned discussion shows that the aim of our examination was to determine cell immunity signs in patients with HPVI CU in whose sera without clinical manifestation anti-HSV-1 and/or HSV-2 of different avidity were revealed by examination of peripheral lymphocytes phenotypic composition.

Materials and methods

43 sick women (average age 26.5 years old) with HPV-induced cervical cancer were examined by colposcopic technique, chain polymerase reaction (CPR), and PAP-test. In scrapes from the cervical canal of UC by CPR DNA 19 high oncogenic – (16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 26, 51, 53, 66, 68, 69, 73, 82) and 9 of weak oncogenic – (6, 11, 40, 42, 43, 44, 54, 61, 70) HPV types were detected. Informed agreement for future examination of sick women was received. Control group consisted of 10 clinically health women (average age 26.5 years old).

Diagnosis of herpetic infection of anogenital area was made by cytological method, PCR and ELISA. Diagnosis of

active herpes was diagnostically correct if there were specific clinical symptoms of disease and positive ELISA in the tested material taken directly from inflammatory foci.

Anti-IgG to HSV-1 and HSV-2 in peripheral blood of sick women were examined by test-system «DIA®-HSV1/2-IgG» (PJSC «SPC Diaproph-Med», Ukraine). After primary sera screening for the presence of anti-HSV-1 and anti-HSV-2 IgG differentiated diagnosis for the presence only anti-HSV-2 by test-system «DIA®-HSV2-IgG-av» (PJSC «SPC Diaproph-Med» Ukraine) which was designed as nondirect solid phase Elisa. As solid phase special polystyrene panels (Sorp (Nunc, Danmark) which were sorbed by cleaned lysed antigen «Herpes Simplex Virus types 1&2» (Virion Ltd.) or protein recombinant mixture of gG1 and gG2 (PJSC «SPC Diaproph-Med», Ukraine). Elisa' carrying out was made according to manufacturer's recommendations.

For examination of serum avidity index, serum were incubated in two parallel wells of the immunosorbent, later one of them was washed by routine mode, and the other was treated with a dissociation solution that causes the destruction of antigen complex with low-avidity antibodies. The avidity index was calculated as the percentage ratio of optical density obtained in the test sample in the presence of a dissociation agent to the optical density obtained by its analysis in the normal mode (regime). If the avidity index was <30 %, it was believed that the serum contained low-avidity antibodies, and in the range of 30 to 60 % – medium avidity ones, and >60 % – high-avidity antibodies.

Phenotypic composition of peripheral blood lymphocytes was examined by laser flow cytofluorimetry. Monoclonal antibodies (Beckman Coulter, USA) for antigens CD3⁺, CD4⁺, CD8⁺, CD3⁺/HLA-DR⁺, CD3⁺/CD16⁺/CD56⁺, CD4⁺45R⁺, CD16⁺/CD56⁺/CD8⁺, and CD16⁺/CD56⁺ were used. The functional status of T cell immune system line was evaluated by the ratio between the helper and suppressor T-lymphocyte subpopulations, immune regulatory index CD4⁺/CD8⁺ was determined (examined?) by this way.

For evaluation of received figures, special computer program STATISTICA was used. The null hypothesis for the control and experimental groups were tested using the non-parametric test of Kolmogorov-Smirnov. The data were represented as M±Std.Dev. The difference was considered statistically significant at P<0.05.

Results and discussion

In CU scrapes in patient with HPV1 papillomaviral DNA mainly of high antigenic risk (16, 18, 31, 33, 35, 39, 45, 51, 56, 52, 58, 59), and sometimes of a low one (6, 11, 42, 43, 44) were identified. According to results of PAP-test in CU in transformation zones, degenerative changes of squamous epithelium, koilocytic atypia, and metaplastic cells were revealed. HPV-induced benign processes of UC, cervical

intraepithelial neoplasia (CIN) of I-II and III degree of activity, and cancer *in situ* were diagnosed in patients. Profuse discharges, itching, and sometimes heartburn, contact hemorrhage were the main clinical complaints of patients with UC HPV infection.

An active herpetic infection of the anogenital area was examined in 3 out of 43 examined persons with UC HPVI (5.0 % of cases), They were excluded from further investigations. The rest examined patients had no clinical symptoms of active anogenital herpetic infection, in UC tested material, obtained from the HPV-transformed zone, HSV DNA were not identified. However, in the blood serum most of them anti-HSV-1 and/ or anti HSV-2 IgG were identified. Thus, anti-HSV-1 IgG were detected in 52.5 % of cases (21 out of 40 patients). In 42.5 % of cases (17 out of 40 patients) anti-HSV-2 IgG only (12.5 %) or simultaneously anti-HSV-2 and anti-HSV-1 (30.0 %) were identified. Serum samples were serologically negative in two patients (5.0 % of cases). These patients were excluded from our further examination, too.

In patients with CU HPV1, anti-HSV-2 IgG with low avidity index were detected in 52.9 % of cases (9 patients out of 17) that is likely to indicate the primary infection with HSV-2 when the contact with virus has occurred relatively recently and the antibodies active centers during immune response have not acquired high degree affinity to the antigen yet. It is necessary to mention that high avidity anti-HSV-2 IgG in blood serum of persons with HPV-infected UC HPV1 were not identified in any case.

Benign processes of UC CIN I-II or CIN III degree of activity, and cancer *in situ* in patients with anti-HSV-1 IgG only were diagnosed in 33.3 % of cases (7 out of 21 patients), 38.1 % (8 out of 21 patients) and 28.6 % (6 out of 21 patients). We observed CIN I-II or CIN III and cancer *in situ*, respectively in 29.4 % of cases (5 out of 17 patients) and 47.1 % (8 out of 17 patients) in patients with either anti-HSV-2 IgG or anti-HSV-2 and HSV-1, while benign processes of UC – in 23.5 % (4 out of 17 patients).

It was established that the incidence of CIN significantly increased in patients with anti-HSV IgG of low avidity. Thus, we observed CIN I-II and III degree of activity and cancer *in situ* in persons with middle-avidity anti-HSV-2 IgG, respectively in 37.5 % of cases (3 out of 8 patients), 25.0 % (2 out of 8 patients), 37.5 % (3 out of 8 patients).

The results of number of main regulatory T-lymphocytes subpopulation and natural killer cells (NK) in peripheral blood of all patients with HPV1 UC are presented in Table 1.

It was established that the absolute and relative amount of CD3⁺ T-lymphocytes, CD8⁺ T suppressors, CD4⁺ T helper cells in peripheral blood, as well as the immunoregulatory index CD4⁺/CD8⁺ were maintained like in the group of clinically healthy persons. At the same time the

amount of CD3⁺/HLA-DR⁺ cells in patients' peripheral blood revealed their significant increase as compared with control group. This observation can confirm both the development of organism inflammatory response and the activation of the T cell immunity. The absolute number of these cells exceeded the upper limit of the norm by 20.0 % and more in 70.6 % of cases (24 out of 34 patients), and the relative one – in 47.1 % (16 out of 34 patients).

In the patients' peripheral blood of control group, there was the absolute and relative number of CD16⁺/CD56⁺ NK (Tabl. 1) and the relative CD3⁺/CD16⁺/CD56⁺ T-lymphocytes number (6.9±4.3) %, but it was (6.2±1.3) %, P>0.05 in

control group. Tendency to the relative number of CD16⁺/CD56⁺/CD8⁺ cells (40.0±14.7) % was observed (in control group –(27.5±9.2) %, P>0.05). However, due to the high patients' individual variability, its difference as compared with the control group was not significant. The content of CD16⁺/CD56⁺/CD8⁺ cells in patients' peripheral blood was higher than the upper limit of the norm by 20.0 % and more in 44.1 % of cases (15 of 34 patients). The relative number of CD4⁺ 45RA⁺ cells in peripheral blood of patients with HPV1 CU did not change in comparison with these data within the control group – (42.3±14.9) %, and (40.5±10.8) %, P>0.05.

Table 1

Phenotypic composition of peripheral blood lymphocytes of clinically healthy persons and patients with HPV1 UC, *M±Std.Dev*

Patient's group	Number of cells in peripheral blood, %					CD4 ⁺ /CD8 ⁺ index
	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD3 ⁺ /HLA-DR ⁺	CD16 ⁺ /CD56 ⁺	
Clinically healthy persons (control)	70.0±2.7 (1.40±0.99)	42.1±2.9 (0.81,0±0.90)	25.2±2.8 (0.46±0.78)	6.3±0.9 (0.12±0.07)	12.5±2.6 (0.20±0.05)	1.7±0.1
HPV-infected persons	70.4±5.1 (1.24±0.35)	41.9±6.5 (0.74±0.25)	25.2±5.9 (0.46±0.16)	18.4±9.2* (0.23±0.05*)	13.6±5.3 (0.22±0.07)	1.8±0.7

Note.* – P<0.05 relatively data in control group. In brackets – cells absolute cells number.

According to individual immunograms, displacement of cells immune response was multi-directional in patients with HPV1 CU whose sera showed IgG antibodies to different types of HSV. As table 2 data represent the relative and absolute number of peripheral blood CD3⁺, CD8⁺, and CD4⁺ cells, as well as the CD4⁺/CD8⁺ immunoregulatory index, did not change in patients with anti-HSV-1 or anti-HSV-2 IgG or anti-HSV-1 and HSV-2 simultaneously, as compared with control.

There was a significant increase of relative number of CD3⁺/HLA-DR⁺ cells (Tabl. 2), which was by 20.0 % higher or more than the upper norm limit in patients which had anti-HSV-1 or HSV-2 IgG antibodies or anti-HSV-1 and

HSV-2 simultaneously, respectively, in 42.1 % of cases (8 out of 19 patients), 60.0 % (3 out of 5 patients) and 54.5 % (6 out of 11 patients). However, the absolute number of these cells increased statistically only in peripheral blood of patients with anti-HSV-1 IgG. There was revealed a tendency to increase CD3⁺/HLA-DR⁺ cells number in the rest of the patients, but the difference as compared with the control group was not significant. Absolute number of CD3⁺/HLA-DR⁺ cells was higher by 20.0 % or more than the upper limit of norm in patients with anti-HSV-1 or HSV-2 IgG at the same time in 68.4 % of cases (13 out of 19 patients), 60.0 % (3 out of 5 patients) and 72.2 % (8 out of 11 patients).

Table 2

Phenotypic composition of peripheral blood lymphocytes in patients with HPV1 UC which contain anti-HSV-1 and/or HSV-2 IgG, *M±Std.Dev*

Patient's group	Number of cells in peripheral blood, %					CD4 ⁺ /CD8 ⁺ index
	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD3 ⁺ /HLA-DR ⁺	CD16 ⁺ /CD56 ⁺	
Clinically healthy persons (control)	70.00±2.70 (1.40±0.99)	42.10±2.90 (0.81±0.90)	25.20±2.80 (0.46±0.78)	6.30±0.90 (0.12±0.07)	12.50±2.60 (0.20±0.05)	1.7±0.1
Patients with anti-HSV-1 IgG	69.20±5.40 (1.22±0.43)	40s.40±6.80 (0.73±0.31)	25.70±5.70 (0.45±0.16)	17.80±9.50* (0.22±0.06*)	14.60±5.20s (0.24±0.08)	1.6±0.6
Patients with anti-HSV-1 and HSV-2 IgG	71.80±5.70 (1.19±0.20)	42.90±6.20 (0.70±0.16)	25.20±6.50 (0.46±0.19)	19.40±9.30* (0.23±0.15)	10.80±4.80 (0.19±0.07)	1.8±0.7
Patients with anti-HSV-2 IgG	72.80±0.90 (1.45±0.12)	43.70±5.80 (0.87±0.15)	26.50±5.80 (0.50±0.14)	18.30±9.60* (0.26±0.14)	13.20±2.10 (0.25±0.05)	1.7±0.5

Note.* – P<0.025 as compared with control data. In brackets – cells absolute number.

In these three patients' groups, the comparison of CD16⁺/CD56⁺ NK number in peripheral blood did not change as compared with control (Tab. 2). Tendency to increase the relative number of CD16⁺/CD56⁺/CD8⁺ cells – (40.4±14.9) %, (45.7±13.0) %, and (40.0±14.7) % was observed, too. In control group it was (26.0±9.2) %, P>0.05). The number of CD16⁺/CD56⁺/CD8⁺ cells in peripheral blood exceeded the upper limit of the norm by 20.0 % and more in patients with anti-HSV-1 or HSV-2 IgG or anti-HSV-1 and HSV-2 IgG at the same time, respectively, in 47.4 % of cases (9 out of 19 patients), 25.0 % (1 out of 4 patients), and 54.5 % (6 out of 11 patients). The number of CD3⁺/CD16⁺/CD56⁺ cells did not change in patients only with anti-HSV-1

or HSV-1 and HSV-2 IgG simultaneously – (9.8±5.9) % and (5.2±2.4) % relatively; in control – (6.2±1.3) %, P>0.05). At the same time there was observed CD3⁺/CD16⁺/CD56⁺ cells number decrease in the patients with IgG antibodies to HSV-2 to (2.4±1.9) % (P<0.05) as compared with control. The relative number of CD4⁺ 45RA⁺ cells in the patients of these three groups was mostly at the level of control.

The relative and absolute number of CD3⁺ CD8⁺ CD4⁺ cells of peripheral blood and the immunoregulatory index of CD4⁺/CD8⁺ in patients with HPV-infected CU, which had anti-HSV-2 IgG of low or medium avidity, were the same as in the control group of patients (Tabl. 3).

Table 3

Phenotypic composition of peripheral T-lymphocytes in patients with HPV CU with low- and medium avidity anti-HSV IgG, *M±Std.Dev*

Patient's group	Avidity of anti-HSV IgG-	Number of cells in peripheral blood, %					CD4 ⁺ /CD8 ⁺ index
		CD3 ⁺	CD4 ⁺	CD8 ⁺	CD3 ⁺ /HLA-DR ⁺	CD16 ⁺ /CD56 ⁺	
Clinically healthy persons	-	70.00±2.70 (1.40±0.99)	42.10±2.90 (0.81±0.90)	25.20±2.80 (0.46±0.08)	6.30±0.90 (0.12±0.07)	12.50±4.00 (0.20±0.05)	1.7±0.1
HPV-infected UC	Low	72.10±4.70 (1.39±0.18)	44.10±4.90 (0.82±0.14)	24.90±5.80 (0.49±0.16)	21.70±8.90* (0.31±0.06*)	10.60±4.30 (0.2±0.08)	1.9±0.6
	Medium	70.90±4.40 (1.15±0.18)	41.50±7.30 (0.68±0.16)	26.00±7.20 (0.46±0.19)	16.80±8.50* (0.19±0.11)	13.00±5.10 (0.21±0.06)	1.8±0.8

The relative number of CD3⁺/HLA-DR⁺ cells increased (Tabl. 2) exceeding the norm upper limit by 20 % or more in 5 out of 7 patients (71.2 % of cases) with low-avidity anti-HSV-2 IgG, and in 3 out of 8 patients (37.5 %) with medium avidity degree of antibodies. However, the CD3⁺/HLA-DR⁺ cells absolute number increased only in patients with low-avidity anti-HSV-2 IgG. In case of medium-avidity anti-HSV-2 IgG, there was only revealed the tendency to the absolute number of these cells increasing. The absolute number of CD3⁺/HLA-DR⁺ cells exceeded the upper limit of the norm by 20.0 % or more in patients with low- or medium-avidity anti-HSV-2 IgG in 85.7 % of cases (6 of 7 patients) and 50.0 % (4 out of 8 patients), respectively. The received facts testify that the number of activated T-lymphocytes in persons with HPV-infected CU considerably occurred during the final stage of acute period of herpes infection when low avidity anti-HSV-2 IgG are revealed.

The CD16⁺/CD56⁺ NK number in patients of these two groups did not relatively change as compared with control (Tabl. 3). In peripheral patients' blood with medium- and low-ability anti-HSV-2 IgG, relative number of CD16⁺/CD56⁺/CD8⁺ cells increased, but the differences has no statistical significance as compared with control (Fig. 1).

However, in case of anti-HSV-2 IgG with low or medium degree of activity, we observed medium avidity increase of these cells number by 20.0 % or more in patients, accordingly in 42.8 % of cases (3 out of 7 patients) and 50.0 % (4 out of 8 patients). The number of CD3⁺/CD16⁺/CD56⁺ cells of patients' peripheral blood with medium avidity anti-HSV-2 did not significantly differ as compared with the control groups of patients (Fig. 2). At the same time the number of these peripheral blood cells considerable decreased in patients with low-avidity anti-HSV IgG as compared with the control group. The relative number of CD4⁺45RA⁺ cells had the same level as compared with the control group in patients with medium and low avidity anti-HSV-2 immunoglobulins (37.6±19.6) % and (43.2±16.9) %, in control group – (40.5±10.8) %, P>0.05).

Our results showed that displacement of cell immunity data, confirmed by number increase of activated CD3⁺/HLA-DR⁺ T-lymphocytes, by tendency to number of CD16⁺/56⁺CD8 cells increasing on the background of significant reduction of CD3⁺/CD16⁺/CD56⁺ cells, was more expressed in patients who had no clinical manifestations of anogenital region of herpetic infection, but had anti-HSV-2 IgG with low degree of avidity. Exactly (just) in these patients

more severe course of HPV-infected CU CMM-HPV-induced CIN I-II and CIN III and *cancer in situ* was diagnosed more often than benign CMM processes. The heavier course of

HPV-CU and proper CIN I-II and CIN III and cancer *in situ* was diagnosed more often than benign CU processes in these patients.

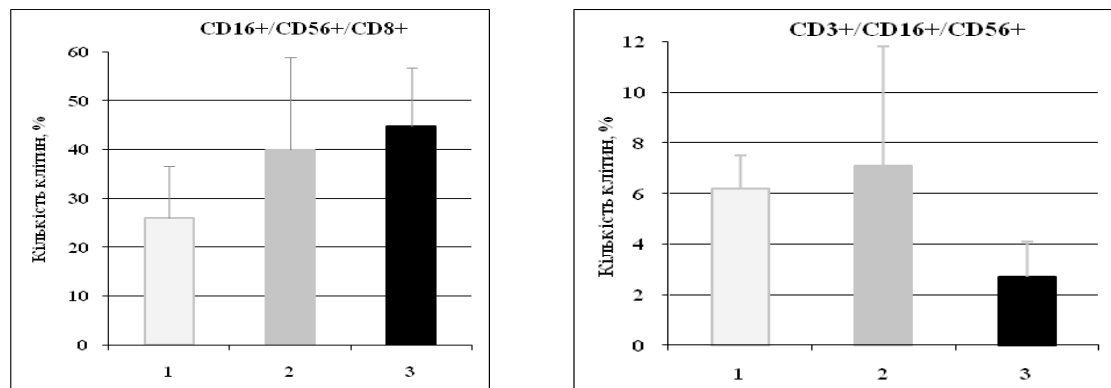


Figure 1. The number of CD16⁺/CD56⁺/CD8⁺ and CD3⁺/CD16⁺/CD56⁺ cells of peripheral blood in patients with anti-HSV-2 IgG of different degree of avidity: 1 – clinically healthy persons (control); 2 – patients with anti-HSV-2 IgG of medium avidity; 3 – patients with anti-HSV-2 IgG of low avidity.

Thus, in the blood serum of most patients with HPV-infected CU, there were anti-HSV-1 or HSV-2 IgG or, anti-HSV-1 and HSV-2 simultaneously, but the clinical manifestation of herpetic infection was diagnosed only in 5.0 % of cases. Giving the results, we took into consideration the fact that partial cross-linked immunity exists between HSV-1 and HSV-2, therefore immunoenzyme «DIA®-HSV2-IgG» test system was used as it creates possibilities to examine (diagnose) anti-HSV-2 IgG with high specificity.

In 42.5 % of cases of HPV-CU, anti-HSV-2 IgG were identified. Among these patients there were those persons who had anti-HSV-1 IgG simultaneously. In accordance with literature data analysis [16], the patients with anti-HSV-2 IgG may be considered potential carriers of this Herpesvirus, and they can suffer from anogenital herpetic infection and they can infect other people. We found out that there was mainly anti-HSV-2 IgG with low degree of avidity (52.9 % of cases) and medium degree of avidity (47.1 %) IgG in seropositive patients with HPV CU serum, whereas high avidity-HSV-2 IgG-2 were never detected. Thus, there was primary infection with HSV-2 in most of patients with HPV CU, although the clinical signs of active anogenital region of herpetic infection were not identified, and the PCR results did not detect HSV-2 DNA (in CU tested material taken from the HPV-transformed zone).

To clarify the atypical course of herpetic infection in patients with HPV CU which was revealed by us as well as to clarify the primary infection with HSV-2 of patients of different comparison groups we determine the phenotypic composition of peripheral blood T-lymphocytes as an important sign of cell immunity.

It should be noted that in the development of the cell immune response in patients with HPV various subpopulations of T-lymphocytes are involved: CD4⁺ and CD8⁺ T cells, CD8⁺CD28⁺, CD8⁺CCR7⁺CD45RA⁺ cells, as well as CD8⁺/CD56⁺ and CD16⁺/CD56⁺ ones [17-19]. Immune control over HPV-induced CIN suppresses CD4⁺/CD25⁺ (high) Tregs with low CD45RA⁺ expression and high expression of CD45RO⁺, GITR, CTLA-4, FoxP3 (forkhead box P3) that have low proliferative activity and low IFN- γ /IL-10 [20]. Immune control over HPV-induced CIN suppresses CD25^(high) Tregs with low expression level of CD45RA⁺ and high expression level of CD45RO⁺, GITR, CTLA-4, FoxP3 (forkhead box P3), which have low proliferative activity and low ratio IFN- γ /IL-10 [20].

We found out that the number of CD3⁺, CD4⁺, CD8⁺, CD4⁺45RA⁺ and CD16⁺/CD56⁺ cells and immunoregulatory index of CD4⁺/CD8⁺ in patients with HPV CU, who had anti-HSV-1 or anti-HSV-2 or anti-HSV-1 and anti-HSV-2 IgG simultaneously were similar at control level. However, the number of CD3⁺/HLA-DR⁺ cells significantly increased, that indicates the activation of T-lymphocytes and can testify both the development of inflammatory response and the formation of an immune response against viral antigens and antigens of atypical cells which are detected in patients with dysplastic CU processes. At the same time in patients with HPV CU, the tendency to the number of CD16⁺/CD56⁺/CD8⁺ cells increase was revealed, that can be a prognostically unfavorable symptom of the disease course. It must be noted that, according to the literature data, the number of CD16⁺/CD56⁺/CD8⁺ cells increase in peripheral blood of patients with HPV I, especially if the number of CD28⁺CD8⁺

cells increase occurs at the same time (some of them can simultaneously have CD28⁺CD8⁺ phenotype), is associated with the persistence of HPV and cervical cancer progression [19], as CD28⁺CD8⁺ cells have both cytotoxic and suppressor functions simultaneously [11].

The displacement of the phenotypic composition of peripheral blood lymphocytes in persons with HPV CU was more expressed primary in patients with anti-HSV-2 IgG with low avidity first of all. We observed not only CD3⁺/HLA-DR⁺ cells number increase and the tendency to CD16⁺/CD56⁺/CD8⁺ cells number increase in these patients but also significant CD3⁺/CD16⁺/CD56⁺ cells number decrease in patients' peripheral blood. It is known that CD3⁺/CD16⁺/CD56⁺ cells have unique spectrum of their activity: ability to cause cytolysis of virus-infected cells and tumor by mechanism independent from MHC, on the one hand, and cytokine-producing activity, on the other hand [21]. Significant increase of the expression of CD3⁺CD56⁺ lymphocytes occurs after patients' vaccination with human papillomavirus vaccine [22]. This fact also confirms an important role of these cells in patient HPV protection. CD3⁺/CD16⁺/CD56⁺ cells number decrease is likely one of the reasons of IF γ production decrease in patients with HPV CU with anti-HSV-2 IgG of low avidity, that may cause cell immunity suppression and cancer progression.

Thus, obtained results indicated that recent HPV CU patients' infection with HSV-2 even without clinical signs of active anogenital region infection, is associated with cell immunity violation, that's why it may be one of the most important risk factors of cells malignant transformation induced by HPV. It confirms that HPV-infected patients with low avidity anti-HSV-2 IgG have more severe cause of disease; CIN I-II and CIN-III, and cancer *in situ* were diagnosed in most cases than in other patients. But addition studies are required to confirm it. It is possible to assert that presence of low-avidity anti-HSV-2 IgG, increase of number of CD16⁺/CD56⁺/CD8⁺ cells and low number of CD3⁺/CD16⁺/CD56⁺ cells in peripheral blood are biological markers which will define individual tactics of complex treatment of patients with HPV CU. It will include immunomodulator and antiviral medicines.

The given study results may be used for determination of character and intensity of immune system alteration in

persons with papillomavirus infection and HPV-induced pre-tumor CU diseases and for improvement of complex individual patients' treatment which may foresee the use of both immunomodulatory and anti-herpetic drugs in case of low-avidity anti-HSV-2 IgG.

Conclusion

1. In patients' blood serum with HPV CU without clinical activity of herpetic infection anti-HSV-1 (52.5 % of cases), anti HSV-2 (12.5 %), and at the same time anti-HSV-1 and HSV-2 IgG (42.5 %) were detected (revealed). In 5.0 % of cases serum samples were seronegative. It was found that anti-HSV-2 IgG in the blood serum of seropositive patients had only medium and low degree of activity that indicates the infection which a person had before as well as primary infection, as a contact with this virus occurred relatively recently and the antibodies active centers haven't had high antigen affinity yet. High avidity anti-HSV-2 IgG in patients' serum with HPV CU weren't identified in any case.

The frequency of detection of HPV-induced dysplastic CU processes of severe degree increased in patients with HSV-2 IgG or with anti-HSV-2 and anti-HSV-1 simultaneously, first of all, in patients with low-avidity anti-HSV-2 IgG.

In patients' peripheral blood with HPV CU where there were detected specific anti-HSV-2 or anti-HSV-1 and anti-HSV-2 IgG, simultaneously, the number of CD3⁺, CD4⁺, CD8⁺, CD4⁺ + 45RA⁺, and CD16⁺/CD56⁺ cells, as well as CD4⁺/CD8⁺ + immunoregulatory index did not change as compared with clinically healthy persons data (control). There was manifested the tendency to the increase of the number of CD16⁺/CD56⁺/CD8⁺ cells as well as a significant increase of activated CD3⁺/HLA-DR⁺ cells, testifying (concerning) T-lymphocytes activation and may reflect the development of inflammatory body response.

Anti-HSV-2 low avidity IgG was identified in patients with HPV CU without clinical manifestation of herpetic infection. They had a more significant displacement of cell immunity confirmed by CD3⁺/HLA-DR⁺ cells number increase as well as tendency to these cell number increase on the background of CD3⁺/CD16⁺/CD56⁺ cytotoxic T-lymphocytes decrease. These facts point out disturbances of patients' cell immunity. Complex personalized treatment of these patients must be reasonably carried out by means of anti-herpetic drugs.

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ФЕНОТИПОВИЙ СКЛАД ЛІМФОЦИТІВ ПЕРИФЕРИЧНОЇ КРОВІ У ХВОРИХ НА ПАПІЛОМАВІРУСНУ ІНФЕКЦІЮ ШИЙКИ МАТКИ, В ЯКИХ ВИЯВЛЯЛИ ІgG АНТИТІЛА ДО ВІРУСІВ ПРОСТОГО ГЕРПЕСУ

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РЕЗЮМЕ. Мета роботи – визначення фенотипового складу лімфоцитів периферичної крові у хворих на папіломавірусну інфекцію (ПВІ) шийки матки

(ШМ), у сироватці крові яких виявляли ІgG антитіла до вірусів простого герпесу (ВПГ)-1 та/або ВПГ-2.

Матеріали і методи. Обстежено 43 жінки (середній вік 26,5 років) із індукованими папіломавірусами людини (ВПЛ) захворюваннями ШМ, діагностованими за допомогою молекулярно-біологічного, кольпоскопічного та цитологічного методів дослідження. Скринінг сироваток на наявність ІgG антитіл до ВПГ-1 та ВПГ-2, а також визначення індексу авідності ІgG антитіл до ВПГ-2 проводили за допомогою твердофазного імуоензимного аналізу. Фенотиповий склад лімфоцитів периферичної крові визначали за допомогою методу проточної лазерної цитофлуориметрії з використанням моноклональних антитіл (Beckman Coulter, США) до

антигенів CD3⁺, CD4⁺, CD8⁺, CD3⁺/HLA-DR⁺, CD3⁺/CD16⁺/CD56⁺, CD4⁺45R⁺, CD16⁺/CD56⁺/CD8⁺ та CD16⁺/CD56⁺. Підрахунок клітин та аналіз результатів проводили на цитофлюориметрі FC-500 (Beckman Coulter, США).

Результати досліджень та їх обговорення. У сироватці крові хворих на ПВІ ШМ, в яких не було клінічних симптомів герпетичної інфекції, виявлено IgG антитіла до ВПГ-1, ВПГ-2 або до цих двох вірусів у 90,0 % випадків. IgG до ВПГ-2 у сироватці крові хворих мали лише середню або низьку авідність. Частота виявлення ВПЛ-індукованих цервікальних інтраепітеліальних неоплазій (ЦІН) I-II та III ступенів і cancer in situ зростала у хворих, в яких були IgG антитіла до ВПГ-2 або одночасно до ВПГ-2 та ВПГ-1, і, насамперед у хворих із низькоавідними IgG до ВПГ-2. У периферичній крові хворих не змінювалась кількість CD3⁺, CD4⁺, CD8⁺, CD4⁺45RA⁺ та CD16⁺/CD56⁺ клітин, а також індекс CD4⁺/CD8⁺ порівняно з показниками для клінічно здорових осіб (контроль). Однак встановлено тенденцію до підвищення кількості CD16⁺/CD56⁺/CD8⁺ клітин, а також суттєве підвищення кількості CD3⁺/HLA-DR⁺ клітин. Кількість CD3⁺/CD16⁺/CD56⁺ клітин зменшувалась у периферичній крові хворих із низькоавідними IgG до ВПГ-2, тоді як у решти хворих зберігалась на рівні контролю.

Висновки. У хворих на ПВІ ШМ, в яких за відсутності клінічної маніфестації герпетичної інфекції ідентифікували низькоавідні IgG антитіла до ВПГ-2, зростала частота виявлення ЦІН тяжчого ступеня та відбувався суттєвіший зсув показників клітинної ланки імунітету, підтверджений підвищенням кількості CD3⁺/HLA-DR⁺ клітин, тенденцією до зростання кількості CD16⁺/56⁺CD8⁺ клітин на тлі зменшення вмісту цитотоксичних CD3⁺/CD16⁺/CD56⁺ Т-лімфоцитів. Імовірно, комплексне персоналізоване лікування цих хворих доцільно проводити з використанням протигерпетичних препаратів.

Ключові слова: папіломавіруси, віруси герпесу, шийка матки, лімфоцити, природні кілери, периферична кров.

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