



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 442-445
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www.thepharmajournal.com
Received: 25-06-2019
Accepted: 27-07-2019

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Informativity of the study of the systemic and local immunity in the diagnosis of the persistent form of latent papillomavirus infection

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Abstract

Data on the role of systemic and local immunity factors that may contribute to the persistence of the human papillomavirus (HPV) in women with latent papillomavirus infection (PVI) of the uterine cervix (UC) are represented in the work. 210 women with PVI were included into the study, of them – 70 women after 6 months had HPV in UC (persistent PVI), the remaining patients were included into the comparison group (transient PVI). The control group consisted of 15 healthy women. Data analysis showed that women with persistent form of latent PVI were characterized by significantly lowered indexes in terms of control of phagocytic neutrophil response, induced NBTR-test of neutrophils and monocytes and monocytes' functional reserve; a significant increase in the percentage of cytotoxic lymphocytes (CD8⁺), a decrease in the immunoregulatory index of CD4⁺/CD8⁺ and serum IgA level; while the number of T-lymphocytes, T-helper cells and natural killers were lowered relative to the norm, but no significant differences were recorded. The state of local immunity in women with persistent PVI differed from the control values with a significantly increased level of sIgA (2.3-fold), IL-10 (2.2-fold), γ -IFN (1.6-fold), and also a low content in the cervical section of the TNF- α and IFN- α (1.3-fold and 1.1-fold, $p>0.05$).

Keywords: persistent latent papillomavirus infection, condition of systemic and local immunity

Introduction

One of the most common sexually transmitted viral etiologies is papillomavirus infection (PVI). PVI is widespread not only in patients with uterine cervix diseases (UC), but also among women who have no changes in the cervical epithelium. Prolonged latent existence of the human papillomavirus (HPV), in which there are no clinical, cytological and colposcopic changes in the UC tissue, can serve as a criterion for an increased risk of cervical pathology [1]. HPV infection and further development of the disease due to the long-term persistence of the virus are largely determined by the cellular and humoral factors of immunity: in PVI there is a decrease of T-cell immunity, functional activity of NK-cells and concentration of the major classes of immunoglobulins [2, 5, 6]. Local immune mechanisms play a key role in preserving the HPV in the body. In particular, cytokine regulation of the immune response to the HPV in the increased probability of the virus persistence manifests itself in the dominance of anti-inflammatory cytokines over pro-inflammatory [3, 8].

The purpose of the work – is to determine the peculiarities of the state of systemic and local immunity in women with persistent form of latent papillomavirus infection of the uterine cervix.

Materials and methods of the research. The study of systemic and local immunity was performed in 210 women of reproductive age (from 18 to 45 years old – mean age 28±0.6 years) with latent form of PVI. The diagnosis is based on the absence of clinical, morphological and cytological changes in the presence of HPV DNA in UC epithelial cells. A control study performed in 6 months revealed that in 70 women, HPV was present in UC (persistent PVI), and other 140 women were a comparison group (transient PVI). The control group consisted of 15 healthy women.

The activity of phagocytosis was determined by the number of peripheral blood cells – neutrophils (NP) and monocytes (MC) in the absorption of latex particles (PN – phagocytic number) with the definition of phagocytic index (PI) (number of latex particles per one

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phagocyte) [4]; oxygen-dependent metabolism of NP and MC – in the Nitro Blue Tetrazolium Reduction Test (NBTR) [12]; taking into account the functional reserve, which was evaluated by the coefficient of activation (Cact.), which was determined as the ratio of the induced test (iNBTR) to the spontaneous one (sNBTR). Cellular immunity was evaluated by the parameters of the relative number of peripheral blood lymphocytes (LC), which were determined by flow cytometry using monoclonal antibodies: CD3⁺ (T-lymphocytes), CD4⁺ (T-helpers), CD8⁺ (T-suppressors/cytotoxic lymphocytes, CTL), CD3⁻/16⁺ (natural killers, NK), CD19⁺ (B-lymphocytes). In blood serum, the level of immunoglobulins (Ig) A, M, G was determined according to the method of Mancini G [10].

The assessment of local immunity (LI) was performed by the level of secretory immunoglobulin A (sIgA), alpha- and gamma-interferon (α- and γ-IFN), tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) in cervical part that was sterile taken from the cervical canal into a centrifuge tube containing 1.0 ml of physiological solution, was homogenized and centrifuged in the regimen of 1500 r/min during 10 minutes, the supernatant fluid was stored until analysis at -20° C. The concentrations of sIgA, α- and γ-IFN were determined by ELISA test using Vektor-Best kits (Novosibirsk, Russia); the level of TNF-α and IL-10 – by means of ELISA sets of the manufacturer “Prokon” (S-Pb, Russia). To unify the obtained results in the study of humoral LI factors, the calculation of a specific concentration per 1 mg of protein in the cervical part was used. The protein concentration in the specimens was determined according to the Bradford’s method [9] using a calibrated curve constructed on human albumin (Sigma-Aldrich, Germany).

Statistical analysis was performed using the application package of programs Statistica 6.0 [7]. The systemic immune parameters were presented as M±m, the LI indeces were

characterized by the median (Me) and the interquartile range in the form of 25 and 75 percent [25%; 75%]. The reliability of the differences between the indeces was evaluated according to the Student’s *t*-criterion and the Mann-Whitney *U*-criterion.

Results and their discussion

In the study of phagocytic activity of blood cells in women with persistent latent form of PVI, the changes in the functional state of NP and MC were revealed, which was expressed in the subsequent inhibition of their phagocytic activity and oxygen-dependent metabolism in relation to similar parameters of the control group (Table 1). Comparing with the data at the beginning of the study, more decreased phagocytic response of NP (phagocyte number (PN) and phagocytic index (PI)) and indicators of spontaneous and induced NBTR-tests of MC. The group of women with persistent PVI differed from the women in the comparison group with a significant reduction in the intensity of NP phagocytosis (at 23.5%) and NBTR-activity of MC of blood – spontaneous and induced tests were reduced in 1.47 and 1.72 times. In the group of women, in whose UC the HPV was not detected after 6 months, the normalization of the functional state of NP and activation of MC was observed, the latter was expressed in an increase of the intensity of the phagocytic reaction of MC and their ability to respond to the additional stimulation (iNBTR); the functional reserve of NP and MC was also restored.

During the study of the cellular link state of the immune system in patients with persistent PVI, an increase of the percentage of CTL (CD8⁺-lymphocytes) and a decrease in the number of T-lymphocytes, T-helper cells, NK and immunoregulatory index (CD4⁺/CD8⁺) in relation to the control was observed.

Table 1: Indexes of systemic immunity in women with latent PVI in the dynamics of observation

Indexes	Healthy donors (n=15)	Periods of examination		
		beginning of the study	in 6 months	
		latent PVI (n=210)	transient PVI (n=140)	persistent PVI (n=70)
PN NP,%	53.7±2.3	51.1±2.2	51.7±2.1	45.6±1.9 *
PI NP,%	6.9±0.7	5.7±0.5	6.8±0.5 ∇	5.2±0.6
sNBTR NP,%	28.6±1.2	37.3±2.4*	29.0±2.1 ♦	26.3±1.8 ♦
iNBTR NP,%	36.6±1.4	41.3±3.0	35.7±2.4	30.2±1.6 *♦
Cact. NP	1.28±0.06	1.11±0.06 *	1.23±0.10	1.15±0.08
PN MC,%	37.2±2.0	34.8±1.4	40.4±1.8 ♦	34.0±1.2
PI MC,%	3.4±0.3	2.9±0.6	4.0±0.5	2.9±0.4
sNBTR MC,%	12.5±1.2	15.4±2.4	15.4±1.3 ∇	10.5±0.8
iNBTR MC,%	20.3±2.0	18.4±1.2	21.8±1.7 ∇	12.7±0.5 *♦
Cact. MC	1.62±0.12	1.19±0.13 *	1.42±0.12	1.21±0.07 *
CD3 ⁺ ,%	67.3±2.0	64.4±2.1	66.1±1.7	63.3±2.4
CD4 ⁺ ,%	39.8±2.3	35.6±2.0	40.6±1.9	36.6±2.5
CD8 ⁺ ,%	27.2±2.1	31.3±1.8	29.6±1.6	32.8±1.3 *
CD3 ⁻ /16 ⁺ ,%	8.8±0.8	10.9±0.9	8.5±1.2	6.7±0.9 ♦
CD19 ⁺ ,%	8.7±0.9	10.7±1.0	8.6±1.0	10.8±1.2
CD4 ⁺ /CD8 ⁺	1.46±0.12	1.14±0.08 *	1.37±0.15	1.11±0.06 *
IgG, g/l	10.3±0.6	11.1±0.9	9.6±1.3	10.5±1.7
IgA, g/l	1.93±0.12	1.90±0.18	2.18±0.31 ∇	1.34±0.07 *♦
IgM, g/l	1.21±0.21	1.66±0.18	1.19±0.15 ♦	1.28±0.09

Notes here and in table 2: significant differences (*p*<0.05) relative to a group of healthy donors (*), women with persistent PMI (∇), ♦ - group of women with latent PVI (beginning of the study)

It should be noted, that natural killers (CD3⁻/16⁺) are an important component of innate immunity, participate in immunological control in malignant neoplasms, as well as in

antiviral defense [13], increase the production of TNF-α and INF-γ. Higher cytotoxic and cytokine-producing activity of NK occur in the presence of HPV [10], stimulation of NK is

associated with the introduction of the virus into these cells with the help of macropinocytosis. Reduction of the number of NK-cells in women with persistent PVI can help prolong the course and complicate the disease. The population composition of blood lymphocytes in women with transient PVI was characterized by an approximation of the number of cells to the control values when compared with the initial data.

In the study of humoral link of the immune system in women with persistent PVI, a decrease in the level of IgA was observed (1.4-fold as compared to the indicator of a group of women with latent PVI and control one and 1.6-fold –in relation to women with transient PVI), at the same time, the level of IgM exceeded the indicator of the comparison group. On the contrary, in blood serum of women with transient PVI there was a decrease in the level of IgM (1.4-fold, $p < 0.05$) and an increase in the level of IgA (1.2-fold) in relation to the general group with latent PVI.

Taking into account the tropism of HPV to a multilayer squamous epithelium, the system of local protection of the reproductive system is important. A special place in the LI has secretory IgA, which provides the protection against viruses (sIgA-antibodies block the attachment of the virus to the cellular wall). The performed studies have shown that women with persistent PVI have increased rates for sIgA of the cervical section (47.0 [32.0; 78.1] $\mu\text{g}/\text{mg}$), as well as women with latent PVI (43.0 [20.0; 78.1] $\mu\text{g}/\text{mg}$), therefore no significant difference was found, however, there were significant differences in the control data (20.4 [11.2; 27.7] $\mu\text{g}/\text{mg}$) and in the women with transient PVI (20.4 [11.2; 27.7] $\mu\text{g}/\text{mg}$) (Fig. 1). An increase in the level of sIgA can be considered as a compensatory mechanism in response to the active intake of new antigens through the epithelial layer that has lost its barrier function [6]. In women with a persistent form of PVI, high levels of sIgA are associated with viral transmission, which holds excessive aggressiveness of free viruses.

A key role in the simultaneous development of inflammatory response and immune reaction to the invasion of a foreign agent is played by $\text{TNF-}\alpha$, which is a positive trigger factor in the chain of cytokine production of the inflammatory cascade and is produced mainly by the normal cervical epithelium. In patients with persistent PVI, there was a decrease in the level of $\text{TNF-}\alpha$ and an increase of the IL-10 level to a greater extent than in women with transient PVI (Table 2). Significant differences for the level of IL-10 were established in comparison with the control group, the group of women with transient and latent PVI, for the level of $\text{TNF-}\alpha$ – only for women with latent PVI of UC. IL-10 provides the implementation of biological effects of T-helpers type 2, which will facilitate the development of humoral component of the immune reaction, which can cause allergic reactivity of the organism. In addition, the excess of IL-10 leads to a decrease in anti-infective protection and chronicity of the process [8]. The decrease of the level of $\text{TNF-}\alpha$ in patients with persistent PVI explains the inhibition of phagocytic function of phagocytes in blood. In women with transient PVI, there was a normalization of the levels of $\text{TNF-}\alpha$ and IL-10 in the cervical part.

The natural recovery of patients in pathological processes will be facilitated by the interferon link of immunity. Research of the content of main interferons in the cervical section in women with latent PVI has determined the increase of both types of IFN concentrations in relation to control (α - 1.4 and γ - 2.1-fold, $p < 0.05$) and decrease of their content after 6 months, but in women with persistent PVI there is a lower level of IFN- α and a higher level of IFN- γ than in women with transient PVI ($p < 0.05$) (Table 2). In women who after six months were determined HPV in the genitals, decrease of median for IFN- γ against women with latent PVI occurred less (1.3-fold) than in women with transient PVI (1.5-fold), and the content of IFN- α in the cervical section decreased more significantly (1.5-fold versus 1.2-fold, respectively).

Table 2: The content of cytokines in the cervical part in women with latent PVI in the dynamics of observation

Indexes per 1mg of protein	Healthy donors (n=15)	Periods of examination		
		beginning of the study	after 6 months	
		latent PVI (n=210)	transient PVI (n=140)	persistent PVI (n=70)
TNF- α , pg	33.8 [20.4; 63.6]	65.2 [34.8; 140.4] *	36.4 [17.9; 69.8] \blacklozenge	26.3 [11.1; 46.3] \blacklozenge
IL-10, pg	21.0 [12.0; 43.0]	34.3 [21.0; 49.4]	23.0 [14.0; 42.0] ∇	47.1 [35.0; 63.7] * \blacklozenge
IFN- δ , pg	39.8 [19.0; 60.5]	55.3 [43.5; 75.5] *	45.5 [31.5; 68.1] $\nabla\blacklozenge$	36.8 [19.0; 44.5] \blacklozenge
IFN- γ , pg	59.9 [42.6; 74.5]	126.4 [75.3; 172.1] *	82.3 [45.3; 126.8] $\nabla\blacklozenge$	94.5 [70.2; 151.5] *

Thus, women with persistent form of latent PVI were characterized by significantly lowered indexes in terms of control of phagocytic neutrophil response, induced NBTR-test of the neutrophil and monocytes and monocytes' functional reserve; a significant increase of the percentage of cytotoxic lymphocytes (CD8^+), a decrease of the $\text{CD4}^+/\text{CD8}^+$ immunoregulatory index and level of the serum IgA, while the number of T-lymphocytes, T-helper cells and natural killers were lowered relative to the norm but no significant differences were noted. The state of the local immunity in women with persistent PVI differed from the control values with a significantly increased level of sIgA (2.3-fold), IL-10 (2.2-fold), γ -IFN (1.6-fold), and also low content in the cervical part of the $\text{TNF-}\alpha$ and IFN- α (1.3 and 1.1-fold, $p > 0.05$).

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