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Peculiarities of tissue immunity in women with various forms of latent papillomavirus cervical infection

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Abstract
Research of indicators of local immunity was performed in 210 women with latent HPV infection (PVI) of the uterine cervix (UC) and in 15 healthy women. 84 women were diagnosed mono-HPV-infection, 126 – were diagnosed combined PVI with other urogenital infections (UGI). It is very important to study peculiarities of tissue immunity in women with various forms of latent papillomavirus cervical infection.

Keywords: latent HPV infection, immune status, lymphocyte phenotyping, mono-HPV-infection, combined PVI.

Introduction

Among sexually transmitted infections, the greatest attention was attracted by papillomavirus infection (PVI), which is the most common not only in women with uterine cervix diseases (UC), but also among women who have no changes in the cervical epithelium, but no one knows the true extent, because only clinical manifestations of PVI, without subclinical and latent forms of HPV-infection, are only registered [4, 16, 18].

Often the infection ends with spontaneous recovery, but in 10-15% of cases the infection becomes chronic, due to the persistence of human papillomavirus (HPV). The possibility of the virus elimination depends on the immune system of the human body; in turn viruses themselves initiate immunodeficiency development leading to long-term persistence and recurrences of the disease [2, 8, 13].

Regarding HPV affinity to stratified squamous epithelium, local protection.

system of reproductive system, whose effectiveness is greatly reduced due to poor HPV antigen expression on the cell surface, is very important. Cytokines play the main role in the regulation of immune response; they represent a large group of factors of intermolecular interactions controlling all successive stages of inflammation and adequacy of response to pathogen invasion, ensure its localization and elimination, and further – reparation of the damaged tissue [14].

Violation of local immune reactions caused by an imbalance of cytokine production in lesion of uterine cervix with human papillomavirus (HPV), may cause long-term persistence of HPV-infection and subsequently initiate the development of cervical dysplasia and increasing severity of genital PVI [5-7]. An important role in the antiviral defense of the organism is given to interferons. According to a number of researchers, PVI develops in the presence of changes in the system of interferons [1].and the violation of the interferon status is at the heart of the pathogenesis of epithelial dysplasia of the uterine cervix [19]. Unfortunately, in PVI of the genital organs of women, the interferon system is not sufficiently investigated yet. Therefore, the study of α- and γ-IFN content in the cervical part is diagnostically feasible for predicting the course of infection Secretory immunoglobulin A (sIgA), the protective effect of which is the ability to inhibit the adhesion of bacteria and virus particles of the surface of epithelial cells of the mucous membranes, without which pathogenic properties are not implemented, plays an important role in the immune defense of the mucous membranes [3]. Most researchers have traditionally noted the changes in indices of local humoral immunity in PVI, emphasize the decrease in sIgA level in cervical mucus, but others indicate an increase of secretion of sIgA in the presence of ectopia in the uterine cervix [10, 17]. Consequently, the participation of secretory IgA in the development and progression of PVI remains unclear.

Discussions on the relationship between PVI and infections of the urogenital tract, caused by conditionally pathogenic flora, still continue. It is believed that bacteria polyamines produced by facultative-anaerobic flora, may have carcinogenic properties and synergistically interact

with HPV, thus dysbiosis of vagina is considered as a potential co-factor of cervical carcinogenesis [9].

Taking into account the above-mentioned, the objective of our research was to study the cytokine and interferon profile and the level of secretory immunoglobulin A in the cervical section of women with different forms of latent papillomavirus infection of the uterine cervix, often associated with urogenital infection, at the beginning of the examination and after 6 months of observation.

Materials and Methods of the Study.

There were studied 1200 women of reproductive age (from 18 to 45 years old – mean age 28±06 years) who applied to the gynecological departments of perinatal centers and hospital №18 in Kyiv for the period from 2011 to 2016. All the patients were performed a comprehensive examination, which included cytological, colposcopic, bacteriological research methods and PCR method. In 210 patients the DNA of HPV was detected in the absence of clinical and morphological signs of infection, indicating latent PVI; of them 84 – were diagnosed monoinfection, 126 – combined papillomavirus and urogenital infections (UGI). Women who were detected UGI received adequate (according to the revealed pathogen) therapy. The control test was performed after 6 months – 140 women had transient PVI, and 70 – persistent one.

Evaluation of local immunity was performed according to the level of secretory immunoglobulin A (sIgA), factor of tumor-alpha necrosis (TNF-α), interleukin 10 (IL-10), interferon-alpha (IFN-α) and interferon-gamma (IFN-γ) in cervical part, which was taken from the cervix with a sterile plastic pipette into a centrifuge tube with 1.0 ml of normal saline solution, was homogenized and centrifuged in mode of 1500 rpm within 10 minutes, the over sediment substance was poured into test tubes type "Ependorf" and was stored until testing at - 20° C. The concentration of sIgA, IFN-α and IFN-γ was determined by enzyme-linked immunosorbent assay (ELISA) using kits "sIgA-ELISA-BEST", "gamma INTERFERON-ELISA-BEST" and "alpha INTERFERON- ELISA-BEST" (Novosibirsk, Russia); the level of TNF-α and IL-10 – with the help of ELISA kits ("Procon", St. Petersburg, Russia).

To unify the results obtained in the study of humoral factors, a calculation of a specific concentration of 1 mg of protein in the cervical part was used. Protein concentration was determined by the method of Bradford [15]. to a sample containing protein 1-10 micrograms in 0.1 ml, 1.0 ml of dye was added – Kumasi brilliant blue G-250, then mixed and measured the spectrum of absorption at 570 nm on a spectrophotometer. The protein concentration was determined using a calibration curve constructed by a human albumin protein (Sigma-Aldrich, Germany).

Statistical processing was performed using Microsoft Office Excel 2003 for Windows and STATISTIKA 6.0 [12]. A comparison of two independent groups was performed using Man-Whitney's non-parametric *U*-criterion. For each metric, the median (Me) and the interquartile spacing of 25 and 75 percentiles (25% and 75%) were determined.

Results of the study and their discussion.

A tumor necrosis factor-α (TNF-α), which is a positive triggering factor in the chain of inflammatory cascade cytokine production stage and is produced mainly by normal cervical epithelium, plays a key role in the simultaneous development of the inflammatory response and immune reaction to the foreign agent invasion.

A significant increase of TNF-α content in cervical part relative to similar values of the control group was observed in women with latent PVI of UC (Table 1).

Table 1: The content of cytokines in cervical part in women with latent PVI

Indeces per 1mg of protein	Healthy women (n=15)	Women with latent PVI, *Me* [25%; 75%]				
		at the beginning of examination (1)			in 6 months (2)	
		totally (n=81)	PVI (n=41)	PVI+ UGI (n=40)	transitory PVI (n=41)	persisting PVI (n=43)
TNF-α, pg	33.8 [20.4; 63.6]	65.2 [34.8; 140.4] *p=0.040	57.7 [29.7; 134.3]	76.6 [37.9; 142.6] *p=0.029	36.4 [17.9; 69.8] ♦p=0.006	26.3 [11.1; 46.3] ♦p=0.004
IL-10, pg	21.0 [12.0; 43.0]	34.3 [21.0; 49.4]	28.7 [14.8; 44.3]	36.0 [24.8; 60.7] •p=0.029	23.0 [14.0; 42.0]	47.1 [35.0; 63.7] *p=0.012 ∇p=0.003 ♦p=0.010

Notes: p<0.05 for healthy women (*); women with HPV monoinfection (•); women with transient PVI (∇); the general group of women with latent PVI (beginning of the study) (♦)

The largest deviations of the TNF-α content from the control values were observed in women with combined PVI and UGI (median was 2.27 times higher, and in case of HPV monoinfection – only in 1.71 times). Re-examination of women with transient PVI of UC in 6 months showed a significant reduction of TNF-α in cervical part relative to the initial data that led to the normalization of statistical

indicators, which could not be said of such factors in the group of patients with persistent PVI of UC, which differed not only from the control and corresponding indicators of a group of women with transient PVI, but also made a significant difference compared with the data of the general group with latent PVI, which were determined at the beginning of the study.

It is known that an increase of TNF-α level can lead to an increase of the expression of the IL-10 gene, which is the most important for its antagonist and normally suppresses the production of pro-inflammatory cytokines. In the early stages of inflammation, IL-10 can inhibit the antimicrobial response, while protecting the body against hyper-inflammation and tissue damage caused by mechanisms of protection against infection. At the same time, IL-10 plays an important role in the pathogenesis of cancer: on the one hand, excessive production of IL-10 increases the probability of tumors; on the other hand, IL-10 inhibits angiogenesis. The analysis of the received data of the determination of IL-10 in the cervical part of women with latent PVI showed an increase of its concentration in terms of the median and interquartile scale

versus the similar values of the control group (Table 2). HPV monoinfection caused an increase of median for IL-10 in relation to control group to a lesser extent (in 1.37 times) than in the presence of additional UGI (in 1.71 times), which was confirmed by significant difference. After 6 months, women who had HPV were observed a further increase in the level of IL-10 in the cervical part, that exceeded twice the median for IL-10 in women with transient PVI, 2.2 times higher than that of healthy women, as well as from indicators, which were received at the beginning of the examination – the median was 1.37 times higher. It should be noted that in women with transient PVI of UC, the values of median, lower and upper quartiles have almost equaled the control ones.

Thus, in women with latent PVI of UC the changes in local synthesis of basic intercontrolled cytokines were marked – the increase of pro-inflammatory TNF-α in cervical part, indicating the activation of the inflammatory response, and at the same time and a slight increase in the level of anti-inflammatory IL-10, which can be considered a compensatory reaction in the first place, at the expense of patients who had combined PVI and UGI. But with the duration of HPV in the uterine cervix the changes that indicated a violation of this cytokine regulation were observed and were manifested in the prevalence of pro-inflammatory cytokines over anti-inflammatory ones. All this may increase the likelihood of the persistence of the virus in the body, and eventually may contribute to the formation of neoplastic processes.

In the case of transient PVI, (that is – when after 6 months, there was no detection of HPV DNA), the concentration of the above-mentioned indicators decreased to almost normal (control) values.

The nonspecific resistance of the organism to viruses normally forms the interferon link of immunity and promotes the natural recovery of patients in pathological processes.

As a result of study the content of IFN-α in the cervical part in women with latent PVI of UC, a significant increase of statistical indices against similar parameters of the control group was found (Table 2). A significant increase in the content of IFN-α in relation to control values was observed in women with combined PVI and UGI, but to a lesser extent (1.31 times) than in HPV monoinfection (1.56-fold), indicating inhibition under the effect of concurrent infection of the synthesis of IFN-α, which is an important antiviral factor in the local protection of the mucous membrane in the presence of pathogens in the genitals. Repeated examination after 6 months has revealed a significant decrease of the IFN-α concentration in the cervical part in women with transient PVI compared to baseline, but its value remained above the control one (Table 2). For women with persistent PVI, the decrease of the IFN-α concentration had a significant difference not only with respect to the baseline but also the median for the group with transient PVI of UC (1.2-fold).

Table 2: The content of α- and γ-interferon in the cervical part of women with latent PVI

Indeces per 1mg of protein	Healthy women (n=15)	Women with latent PVI, *Me* [25%; 75%]				
		at the beginning of examination (1)			in 6 months (2)	
		totally (n=81)	PVI (n=41)	PVI+ UGI (n=40)	transitory PVI (n=41)	persisting PVI (n=43)
TNF-α, pg	39.8 [19.0; 60.5]	55.3 [43.5; 75.5] *p=0.002	62.3 [43.0; 76.0] *p=0.003	52.3 [45.0; 74.2] *p=0.006	45.5 [31.5; 68.1] ♦p=0.011	36.8 [19.0; 44.5] ∇p=0.018 ♦p=0.009
IL-10, pg	59.9 [42.6; 74.5]	126.4 [75.3; 172.1] p=0.0001	123.0 [75.3; 162.3] *p=0.0003	128.1 [79.5; 174.1] *p=0.0004	82.3 [45.3; 126.8] ♦p=0.0005	94.5 [70.2; 151.5] *p=0.0007 ∇p=0.021

Notes: p<0.05 for healthy women (*); women with transient PVI (∇); the general group of women with latent PVI (beginning of the study) (♦)

Analysis of the data as for the content of IFN-γ showed its significant increase in cervical part of women with latent PVI relative to control (Table 2) and equally both in women with HPV monoinfection and with combined PVI and UGI. Increase of IFN-γ, which has direct antiviral activity and being an important immune regulator that enhances the synthesis of HLA-antigens by cells, can result in women with latent PVI in acceleration of the processes of recognition and processing of antigens, activating other immune cells that participate in the elimination of pathogens. Perhaps because of this, 6 months later most women were not detected DNA to HPV. Repeated examination in women with transient PVI showed a significant decrease in the concentration of IFN-γ in respect to initial data, although the level of IFN-γ remained higher than normal, which may indicate the activation of T-helpers of type 1 and natural killer cells. In the group of patients with persistent PVI median for IFN-γ was significantly higher than the same indicator of the group of women with transient PVI (in 1.14 times) and control group (in 1.58 times). This fact indicates that IFN-γ, which is capable of providing a cytokine cascade, in the presence of persistent PVI supports the formation of an inflammation center with the involvement of effector cells.

Thus, the increase of the content of basic interferons α and β in women with latent PVI of UC in cervical part is a response to genital infections not only with human papillomavirus, but also with other pathogens. Deficiency of IFN-α, which was found in the group of women with persistent course of PVI, may be due to both disturbances in the functioning of the immune system and the ability of high-risk HPV to inhibit the synthesis of interferons and interferon-signaling pathways work and thus contributes to the long-term persistence of HPV.

Secretory immunoglobulin A has a special place in local immunity. The exceptional importance of sIgA-antibodies in antiviral defense is due to the fact that they are from the very beginning present in the places of primary contact of the virus with the epithelial cells of the mucous membranes of the host's organism. The performed studies showed almost twice (p=0.003) increase of median and interquartile range (43.0 [20.0-78.1 μg/mg]) for the content of sIgA in the cervical section of women with latent PVI compared to healthy persons (20.4 [11.2, 27.7] μg/mg). Probably, the increase of secretory IgA is due to the fact that it is an important compensatory reserve of the organism that provides the protection against viruses and promotes the elimination of

pathogens, since high concentrations of sIgA-antibodies block the attachment of the virus to the cellular wall. The presence of UGI in women with PMI of UC contributed to a further increase of the median for sIgA (2.3 times), than in patients with only PVI (2.0-fold). There were determined significant differences as for the control group of women with mono-PVI (41.1 [17.2; 78.1] μg/mg, p=0.008) and combined with UGI (47.7 [23.8; 79.2] μg/mg, p=0.003).

A significant decrease of sIgA concentration up to 25.6 [14.0; 53.2] μg/mg (p=0.005) in relation to the initial values, which was slightly different from the control values occurred after 6 months in women with transient PVI of UC. In patients who were detected HPV in the genitals, an elevated level of secretory IgA (47.0 [32.0; 78.1] pg/mg) was noted, as at the beginning of the study, thus the statistical difference from the initial data was not true, but there were determined the significant differences relative to the control data (2.3-fold, p=0.005) and index of the group of women with transient PVI (1.8-fold, p=0.003). In this case, in our opinion, high levels of sIgA accompany the virus-carriage, restraining excessive aggressiveness of free viruses.

Thus, the increase of sIgA in cervical part of women with latent PVI and in combination with UGI can be considered as a compensatory mechanism in response to an active input of new antigens through the epithelial layer, which lost its barrier function, as it is evidenced by the research results of other authors [11]. The movement to normalize the level of secretory immunoglobulin A was observed only in women whose HPV disappeared after six months.

Therefore, the performed studies have found that an imbalance of cytokine regulation of the local immune response with a prevalence of anti-inflammatory cytokine IL-10 amid a backdrop of the decrease of the level of pro-inflammatory cytokine TNF-α and antiviral α-IFN with preservation of a high level of sIgA and γ-interferon may be important for maintenance of the HPV persistence in the uterine cervix and further contribute to chronization of infectious-inflammatory process.

Conclusions

1. The tension of local immunity in women with identified HPV in the uterine cervix was characterized by a significant increase in the cervical section of the content of secretory IgA (2.10 times), TNF-α (1.93 times), α- and γ-IFN (in 1.39 and 2.11 times) compared to healthy women.
2. It is proved that mixed infection of genital tract in women with cervical latent PVI caused the greater severity of changes in local immunity compared with the control values that was manifested in the larger than in HPV monoinfection increase of content of TNF-α and IL-10 (in 2.27 and 1.71 times versus 1.71 and 1.37) and oppression of α-IFN synthesis (1.31 versus 1.56).
3. The condition of local immunity in women with persistent form of PVI was characterized by a significantly increased level of sIgA (1.8 times), IL-10 (2.0 times), γ-IFN (1.14 times), and a reduced level of α-IFN (1.2 times) compared to women with transient form of PVI.

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