DOI: http://doi.org/10.17816/2313-8726-2021-8-3-155-166

# Efficacy of immunological antivirus therapy for papillomavirus-associated grade I cervical intraepithelial neoplasia

Ol'ga P. Vinogradova[1], Natal'ya A. Andreeva[2], Ol'ga V. Epifanova[1], Ol'ga I. Artemova[3]

[1]Penza Institute of Advanced Training of Doctors — Branch of the Russian Medical Academy of Postgraduate Education, Penza, Russian Federation;
[2]Ogarev Mordovia State University, Saransk, Russian Federation;
[3]Scientific Research Institute of Fundamental and Applied Research of the Penza State University, Penza, Russian Federation

***ABSTRACT***

***AIM:*** The study aimed to assess the effectiveness of alloferon in human papillomavirus (HPV)-associated cervical neoplasia (grade I) based on the analysis of the cytokine profile in cervical mucus as well as markers of apoptosis in cervical epithelial cells.

***MATERIALS AND METHODS:*** The study enrolled 98 women, including 55 women of reproductive age with cervical intraepithelial neoplasia (CIN) associated with HPV infection and 43 conditionally healthy women without HPV infection. Factors of cytokine response and markers of apoptosis under normal and pathology conditions were assessed and compared.

***RESULTS:*** The imbalance of pro-inflammatory and anti-inflammatory cytokines, in favor of the latter, is an important factor that supports the persistence of HPV-associated grade I CIN. Reducing caspase-3 and caspase-9, increasing interleukin-18, and subsequent activation of interferon gamma against the background of alloferon use are favorable signs of substantial elimination of the HPV.

***CONCLUSIONS:*** The results of this study show considerable elimination of HPV in patients with grade I CIN when using immunological antivirus therapy.

**Keywords:** papillomavirus infection; cervical intraepithelial neoplasia; cytokines; interleukin-18; alpha tumor necrosis factor; interferon gamma; caspase; human papillomavirus; viral load alloferon; cervical cancer; polymerase chain reaction; genotyping; serious oncogenic risk; allokin alpha.

**To cite this article:**
Vinogradova OP, Andreeva NA, Epifanova OV, Artemova OI. Efficacy of immunological antivirus therapy for papillomavirus-associated grade I cervical intraepithelial neoplasia. *V.F. Snegirev Archives of Obstetrics and Gynecology.* 2021;8(3):155–166. (In Russ).
doi: 10.17816/2313-8726-2021-8-3-155-166

**Received:** 04.05.2021 **Accepted:** 15.07.2021 **Published:** 15.09.2021

DOI: http://doi.org/10.17816/2313-8726-2021-8-3-155-166

# Эффективность применения иммунопротивовирусной терапии цервикальных интраэпителиальных неоплазий I степени на фоне папилломавирусной инфекции

О.П. Виноградова[1], Н.А. Андреева[2], О.В. Епифанова[1], О.И. Артёмова[3]

[1]Пензенский институт усовершенствования врачей — филиал Российской медицинской академии последипломного образования, Пенза, Российская Федерация;
[2]Национальный исследовательский Мордовский государственный университет им. Н.П. Огарёва, Саранск, Российская Федерация;
[3]Научно-исследовательский институт фундаментальных и прикладных исследований Пензенского государственного университета, Пенза, Российская Федерация

***АННОТАЦИЯ***

***Цель*** — оценка эффективности применения аллоферона при ассоциированных с вирусом папилломы человека (ВПЧ) цервикальных неоплазиях I степени на основании анализа цитокинового профиля в цервикальной слизи, а также маркеров апоптоза в эпителиальных клетках шейки матки.

***Материалы и методы.*** В исследование вошли 98 женщин, из них 55 женщин репродуктивного возраста с цервикальной интраэпителиальной неоплазией (Cervical Intraepithelial Neoplasia — CIN), ассоциированной с вирусом папилломы человека (ВПЧ), и 43 условно здоровые ВПЧ-негативные женщины. Проведена сравнительная оценка факторов цитокинового ответа и маркеров апоптоза в норме и при патологии.

***Результаты.*** Дисбаланс провоспалительных и противовоспалительных цитокинов в пользу последних — важный фактор, поддерживающий персистенцию вируса папилломы человека в эпителии шейки матки при цервикальной интраэпителиальной неоплазии I степени. Снижение показателей каспазы-3 и каспазы-9, повышение интерлейкина-18 и, как следствие, активация интерферона-гамма (IFN-γ) на фоне применения аллоферона — благоприятные признаки, способствующие высокой степени элиминации вируса папилломы человека.

***Заключение.*** Полученные в ходе проведённых исследований данные свидетельствуют о высокой степени элиминации папилломавирусной инфекции у пациенток с цервикальной интраэпителиальной неоплазией I степени на фоне применения иммунопротивовирусной терапии.

**Ключевые слова:** папилломавирусная инфекция; цервикальная интраэпителиальная неоплазия; цитокины; интерлейкин-18; фактор некроза опухоли альфа; интерферон-гамма; каспазы; вирус папилломы человека; вирусная нагрузка; аллоферон; рак шейки матки; полимеразная цепная реакция; генотипирование; высокий онкогенный риск; аллокин-альфа.

**Как цитировать:**
Виноградова О.П., Андреева Н.А., Епифанова О.В., Артёмова О.И. Эффективность применения иммунопротивовирусной терапии цервикальных интраэпителиальных неоплазий I степени на фоне папилломавирусной инфекции // Архив акушерства и гинекологии им. В.Ф. Снегирёва. 2021. Т. 8, № 3. С. 155–166. doi: 10.17816/2313-8726-2021-8-3-155-166

**Рукопись получена:** 04.05.2021 **Рукопись одобрена:** 15.07.2021 **Опубликована:** 15.09.2021

In routine clinical practice, difficulties in managing patients with cervical intraepithelial neoplasia (CIN) are associated, first, with the lack of a standard treatment and diagnostic and preventive measures for cervical precancer, and second, with a lack of understanding of the processes by which human papillomavirus (HPV) induces squamous intraepithelial lesions, the mechanisms of action of anti-HPV drugs and, as a result, irrational methods of influencing cervical tissue.

In this regard, it seems relevant to develop and clinically test a method that utilizes the etiopathogenetic effect on the initiating factors and cofactors of oncogenesis to create an optimal radical effect in order to achieve complete regression of the pathological focus. This will reduce the incidence of cervical tumor transformation in women of reproductive age, as well as improve the results of treatment and enhance the quality of life of patients.

However, until recently, the model for studying the mechanisms of cervical carcinogenesis and CIN development remains quite unique and is frequently discussed among other oncogynecological issues [1]. When HPV enters the human body in the early stages, the processes of recognition the infectious agent, inflammation, clearance, and cell death are interconnected due to the control action of the innate immune system. Among the range of endogenous modifying factors responsible for the genesis of cervical epithelium malignancy, local immune mechanisms are significant, particularly cytokine regulation of the local immune response to HPV [2, 3]. A complex of lymphoid structures, phagocytic cells, including macrophages of cervical stromal tissues, and secretory humoral and cytokine factors provide local antiinfective protection.

Cytokines, which represent a wide group of intermolecular interaction factors, play a major role in the regulation of the immune response. These include interferons (IFN), interleukins (IL), and growth factors [4, 5]. It has been proven that IL-18, which is a pro-inflammatory cytokine, independently (FasL) or through IFN-γ (Fas) stimulates the initiation of apoptosis. Fas/FasL is a receptor-ligand system underlying one of the main receptor-dependent pathways of apoptosis. Natural killers (NK cells) are the fundamental factors of antiviral protection in the early stages of immune processes, and they implement rapid cytolysis after HPV penetration. Although NK cells implement a cytotoxic reaction without an extensive immune response, they require preliminary activation regulated by the cytokines IL-2, IL-15, and IL-18 [6].

The cytoplasm of NK cells contains granules with the protein perforin (cytolysin), which plays the main role. Due to stimulated exposure, the contents of the intracellular granules of NK cells are released into the extracellular space after HPV penetration [7, 8]. Perforin is incorporated into the target cell membrane and forms transmembrane pores, which induces cell death as the cell contents leak out through these pores, thereby initiating apoptosis.

In addition, IL-18 is able to stimulate the production of IFN-γ. The properties of IFN-γ include the induction of antiviral protection and antiproliferative action due to the activation of cells of the monocyte–macrophage system and an increase in their ICE activity (ICE is an IL-1-converting enzyme) [5].

Tumor necrosis factor α (TNF-α) is one of the main representatives of the TNF-α family of cytokines. It is an inducer of apoptosis and is recognized by receptors similar in structure to Fas. This group of cytokines has both antitumor cytotoxicity and inflammatory and immune reactivity effects. TNF-α is the main external mediator of apoptosis. Some authors have established that tumor necrosis factor secreted by monocytes, macrophages, and T-lymphocytes triggers an intracellular cascade of caspase activation [9, 10].

Intracellular proteins and enzymes associated functionally with clusters of differentiation play a significant role in the mechanisms inducing apoptosis under the influence of the transforming action of HPV. Cellular caspases (caspase is a cystein aspartate-specific protease) are central mediators and effectors of apoptosis responsible for the intracellular propagation of the apoptotic signal and the final implementation of the apoptotic program. The process of apoptosis is multistage and accompanied by a cascade of caspase reactions [11]. The process of programmed cell death can proceed in two ways, external and internal. One of the key links in the internal mitochondrial pathway is caspase 9, which is activated by its precursors after cell DNA damage. Regardless of the pathway through which apoptosis is induced, its final stage is the activation of effector caspase 3. Violation of interactions at any stage of apoptosis can lead to various consequences, ranging from impaired elimination capabilities to progression to atypia.

There are currently no studies on cytokines changes and caspase expression in CIN development, as well as data evaluating the levels of caspases and their activity in cervical epithelial cells at different stages of cervical cancer (CC) development.

This study aimed to evaluate the efficiency of using Alloferonum in HPV-associated (CIN I) based on analysis of the cytokine profile, as well as markers of apoptosis in the cervical mucus of these patients.

## MATERIALS AND METHODS

A study was conducted on 98 women who presented for an outpatient visit in the city of Penza from 2017 to 2019. In the study, the female patients were distributed into two groups: Group 1, comprising 55 patients with confirmed HPV-positive cervical intraepithelial lesions grade 1; Control group, comprising the remaining 43 women with a normal cytological presentation, without HPV infection and, accordingly, had the NILM category according to oncocytology. Examination of the control group provided the results of the physiological norm of the parameters under study.

The women examined were aged 18 to 49 years, and the average age of women in the CIN I and control groups was 27.05±0.51 years and 30.41±1.07 years, respectively.

The study was approved by the local ethics committee of the Penza Institute for Postgraduate Medical Education, a branch of the Russian Medical Academy of Postgraduate Education (No. 12 of December 17, 2018), and the patient informed consent forms were also approved. Informed consent was obtained from all patients for participation in the study and publication of their medical data.

To develop new approaches for treating human papillomavirus infection, cytokine response factors and apoptosis markers were evaluated in women with grade 1 CIN associated with HPV infection (group 1), and their changes were compared with those of conditionally healthy women without HPV (control group), who had normal oncocytological smear parameters from the cervix and a normal colposcopic presentation.

The inclusion criteria for Group 1 were the presence of high-risk HPV virus replication in the cervical canal, as detected by polymerase chain reaction (PCR); histologically confirmed CIN I; the absence of therapy with drugs that could influence the results of the study during the last 6 months before the start of the study; adequate contraception in women of childbearing age (use of a barrier method of contraception); written informed voluntary consent of the patient to participate in the study and use her medical data; type 1 and 2 transformation zone; "normocenosis" according to the results of the assessment of the lower gynecological tract; and patient compliance.

Exclusion criteria included age under 18 years and over 49 years; pregnancy; lactation; severe somatic pathology; the intake of drugs that could affect the parameters studied; the presence of decompensated diseases or acute conditions, including concomitant mental illnesses; the presence of other sexually transmitted infections; and inability to follow the protocol.

Withdrawal criteria from the study were taken into account, that is, the appearance of exclusion criteria during the study, individual intolerance to the drug during the study, non-compliance with the regimen of taking the drug, and the patient's decision to withdraw from the study.

To assess the cellular composition and identify atypical epithelial cells, a cytological study of smears from the cervix of the examined women was performed. The material for cytological examination was a swab from the cervical canal, transformation zone, and surface of the cervix, obtained using a disposable cervical brush. Cervical smears were stained according to Papanicolaou. The results of the cytological study were evaluated according to the general provisions of the Bethesda informative classification system developed in the USA in 1988 [12].

Detection and differentiation of HPV DNA was performed by PCR with hybridization-fluorescence detection of AmpliSense® HPV high carcinogenic (HRC) screen-titer-FL, according to the manufacturer's recommendations (Central Research Institute of Epidemiology, Moscow). The set of reagents was designed to detect and quantify HPV DNA of high HRC including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 in clinical material. The method is based on the simultaneous amplification of HPV DNA regions and a DNA region of the β-globin gene, which was used as an endogenous internal control, with endpoint hybridization-fluorescence detection (FEP format). Scraping discharge from the cervical canal was used as the material for PCR diagnostics. The final diagnosis was made using a multifocal biopsy from suspicious areas identified during a colposcopic examination.

The local immune status was investigated during the collection of cervical mucus, and the material for determining the levels of caspase 3 and caspase 9 was cell scraping from the cervical canal, which was obtained using a disposable cervical brush. Cytokines were determined by competitive enzyme immunoassay (ELISA) using the Vector-BEST reagent kit (Novosibirsk). The results were recorded according to data from the standard calibration curve, followed by the calculation of the cytokines concentration in the studied samples. The results were expressed in pg/ml.

To determine the expression levels of caspase 3 and caspase 9, a kit of reagents from Cloud-Clone Corp., designed for the quantitative determination of CASP3 by a sandwich ELISA method in tissue homogenates, cell lysates, cell culture supernatants, and other human biological fluids, was used.

Statistical analysis of the data was performed using evaluation methods and the STATISTICA 9.0 program. Descriptive statistics were also used to summarize the data. statistical analysis of the indicators was performed using the Fisher angular transformation method. The difference between the compared data was considered significant at $p < 0.05$.

## RESULTS AND DISCUSSION

Analysis of the immunological parameters of interest in the cervical mucus of patients with CIN I associated with HPV infection revealed common patterns when compared with each other, as well as with the control group (Table 1).

Although IFN-γ has a direct antiviral and immunostimulatory effect, under certain circumstances, it can be an immunosuppressant and a factor contributing to HPV persistence [2]. Several authors noted that activation of HPV IFN gene expression leads to an increase in IFN-γ concentration in the blood of patients infected with HPV [13]. The highest activation of the IFN system was triggered by HPV types with a high oncogenic risk [14].

A significant difference in the mean IL-18 level was found in the group with CIN I (8.97±0.12 pg/ml) compared with the control group (8.12±0.07 pg/ml).

It should be noted that in patients with CIN I, when compared with a group of healthy women ($n$=43), changes in local

**Table 1.** Cytokine levels in the cervical mucus in the examined groups of women, M±m

| Study group | IFN-γ, ng/ml | IL-18, ng/ml | TNF-α, ng/ml |
|---|---|---|---|
| CIN I (*n*=55) | 123.85±1.66** | 8.97±0.12* | 2.76±0.05 |
| Control group (*n*=43) | 89.44±0.85** | 8.12±0.07* | 2.66±0.05 |

*Significant difference between both groups (Mann–Whitney test, $p < 0.05$); **tendency toward a significant difference.

protection parameters were characterized by an increase in the concentrations of IFN-γ, IL-18, and TNF-α.

There was no significant difference in age of the patients in both groups (*p*=0.47).

According to the proposed study design, we subsequently analyzed the markers included in the study to assess the severity of apoptosis, i.e., the activity of caspase 3 and caspase 9 in cervical epithelial cells was determined. However, we could not find the norms for caspase 3 and caspase 9 obtained by the ELISA technique.

In this regard, to determine the norm values of these parameters, patients from the control group were examined, and then the activity of caspase 3 and caspase 9 was determined in the group of patients with HPV-associated CIN I (Table 2).

Due to apoptosis in healthy cells for the regulation of normal processes, the values obtained for the markers in the control group indicate the presence of natural death, through which the regulation of cell renewal in the cervical zone occurs.

When determining the levels of caspase 3 and caspase 9, the parameters in the group with CIN I differed significantly from those in the control group. The change in the activity of apoptosis markers in the group of HPV-positive patients with CIN I compared to the control group indicates the effect of HPV on cervical epithelial cells by stimulating apoptotic processes. To activate the process of programmed cell death via an external or internal pathway, a trigger must act on the cell receptors; in this case, HPV plays such a role. Thus, the results obtained suggest that HPV, penetrating the cell, even in its episomal form, can influence the vital activity of the cell due to the activation of death receptors on the outer membrane and by affecting the mitochondria. These interactions lead to an increase in apoptosis as a protective reaction of a damaged cell, that is, in HPV-associated CIN I, apoptosis is increased due to continuous stimulation of epithelial cell receptors.

In the next stage of the study, all patients with CIN I (group 1) were distributed into two subgroups of equal size:

- subgroup 1a included 28 patients with CIN I who received therapy with Alloferonum (Allokin-alpha) according to the protocol;
- subgroup 1b included 27 patients with CIN I who were under follow-up without the use of therapy.

There was no significant difference in age of the patients in both subgroups (*p*=0.5941).

The control group was not divided into subgroups.

Alloferonum (Allokin-alpha) is an oligopeptide that activates the natural killer system, capable of stimulating the recognition and lysis of defective cells by cytotoxic lymphocytes. In addition, Alloferonum induces the synthesis of endogenous interferons, mainly IFN-γ, activates cytotoxic $CD3^+HLA\text{-}DR+$ T cells even against the decrease in the absolute count of CD3+CD8+ cells, which is important for the implementation of the antitumor and antiviral response.

After the administration of Alloferonum, the following effects are noted.

1. An increase in IFN-γ synthesis and a 37-fold and 32-fold increase in its concentration in the cervical mucus compared with the initial level and the control group, respectively [15].

IFN-γ activates the effector functions of neutrophils, macrophages, cytotoxic T-lymphocytes, and NK, since these cells have receptors for this interferon. They increase cytotoxicity, microbicidal activity, and the production of cytokines, nitrooxide radicals, and superoxide radicals, which leads to the death of intracellular parasites, including viruses.

In addition, IFN-γ inhibits the anti-inflammatory IL-4 and B-cell response but enhances the production of pro-inflammatory IL-2, which stimulates the proliferation of T-killers.

IFN-γ increases the expression of major histocompatibility complex antigens of classes 1 and 2 on various cells, and induces the expression of these molecules even on cells that do not express them constitutively. This leads to an increase in the efficiency of antigen presentation and the ability of T-lymphocytes and NK to recognize antigens.

2. A 24-fold increase in the concentration of IL-1β compared with the initial level [15].

This cytokine can induce NO synthases, thereby increasing the production of nitric oxide by phagocytes, which is involved in the processes of phagocytosis.

3. A 3.4-fold and 2.7-fold increase in the concentration of nonspecific enterase compared with the initial level and the control group [15]. An increase in macrophage activity

**Table 2.** The level of caspase 3 and caspase 9 in the study groups — control and CIN I

| Study group | Caspase 3, ng/ml | Caspase 9, ng/ml |
|---|---|---|
| Control group (*n*=43) | 0.18±0.04 | 0.21±0.03 |
| CIN I (*n*=55) | 2.27±0.03* | 2.31±0.05* |

*Caspase activity differed significantly between the groups (according to Wilcoxon–Mann–Whitney criteria, $p < 0.05$).

also illustrates an increase in the concentration of myeloperoxidase.

Nonspecific enterase is a cytoplasmic enzyme of dendritic cells and T-killers; therefore, it can be concluded that their activity is also proportionally increased.

4. In the complex treatment of severe dysplasia of the cervical epithelium and CC, the use of Alloferonum leads to a significant decrease in the levels of the immunosuppressive proteins TGF-β and FOXP3, which block the activation of lymphocytes and macrophages, and also enhance angiogenesis in the tumor.

It should be emphasized that these changes develop at the location of the infectious agent or tumor tissue, and not systemically.

The most interesting general immune effect resulting from the use of Allokin-alpha is an increase in CD4 concentration from 32.8% to 50.54% by days 12–18 from the start of treatment and correction of the CD4/CD8 immunoregulatory index from 1.16 to 2.00 [16].

After the treatment, all patients underwent strict follow-up, including clinical and cytological examination, as well as extended colposcopy and HPV testing (HPV testing was performed 3, 6, and 12 months after the treatment).

Due to the availability of multiple evidence that the antigen dose can affect the activity and nature of the immune response [12], we investigated the relationship between HPV concentration in cervical scrapings and indicators of local and systemic immunity in women with CIN I before and after treatment.

HPV viral load changes were noted. Therefore, among patients in the CIN I (1b) case follow-up subgroup, 2 (7%) patients showed a decrease in viral load after 3 months, and 4% of patients did not have HPV infection. In subgroup 1a, viral load decreased in 14%, and HPV was not detected in 7% of patients. Thus, the most favorable results after 3 months were recorded in patients who used antiviral therapy. When analyzing the results obtained, a significant difference was noted between the subgroups after 3 and 12 months (Table 3).

When analyzing the viral load changes in the subgroups after 6 months, the following results were obtained. In subgroup 1b, 15% of patients had values below 3 Ig, and the virus was not detected in 7% of cases. In subgroup 1a, 10% of patients had a lower concentration of the virus, and the virus was not detected at all in 21% of patients.

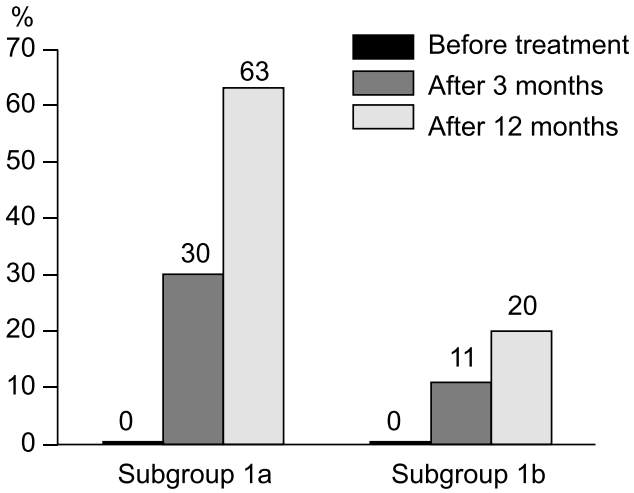


**Fig. 1.** Evaluation of the effectiveness of therapy in the study subgroups.

After 12 months, in subgroup 1b, the viral load decreased below the clinically significant level (less than 3 Ig) in 4% of patients, and the virus was not detected in 11% of cases. In subgroup 1a with antiviral treatment, 11% of women had a decrease in viral load, and HPV was not detected in 29% of women after 12 months.

For the convenience of data interpretation, the concept of "efficacy" was adopted, which was defined as the total indicator of the absence of viral load and a decrease below a clinically significant concentration (Fig. 1).

Figure 1 shows that a decrease in viral load and/or complete elimination of HRC HPV in the subgroup that received treatment was noted in 30% of women after 3 months and 63% of women after 12 months, whereas in the patients of the other subgroup under case follow-up, this indicator after 12 months did not exceed 20%.

Based on the analysis of the data obtained, it was found that the patients in subgroup 1b with re-diagnosed HPV infection were of the older age group (41–49 years old), had bad habits including smoking more than 10 cigarettes a day, and a loaded obstetric history with 2 or more abortions.

Distinctive aspects of the cytokine profile of patients with CIN I from subgroup 1a, 10 days after treatment with Alloferonum, were a noticeable increase in the level of IL-18 from 8.82 ± 0.21 to 12.61 ± 0.35 pg/ml. After 12 months of follow-up in subgroup 1a, the level of IL-18 decreased insignificantly, but remained higher than the initial value.

**Table 3.** Comparison of indicators in patients of subgroups with CIN I

| Indicator | CIN I, subgroups, *M±m* | |
|---|---|---|
| | 1a | 1b |
| Viral load of Ig HPV/$10^5$ epit. cells before treatment | 4,48±0,10 | 4,47±0,10 |
| Viral load of Ig HPV/$10^5$ epit. cells after 3 months | 4,52±0,09 | 4,51±0,09 |
| Viral load of Ig HPV/$10^5$ epit. cells after 12 months | 3,31±0,26* | 4,25±0,21* |

*Significant difference between the subgroups (Mann–Whitney test, $p$ <0.05).

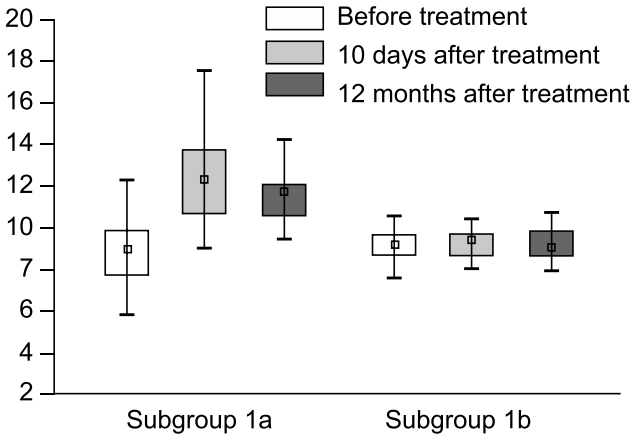


**Fig. 2.** The concentration of IL-18 in the cervical mucus of the studied groups of women with CIN I, pg/ml.

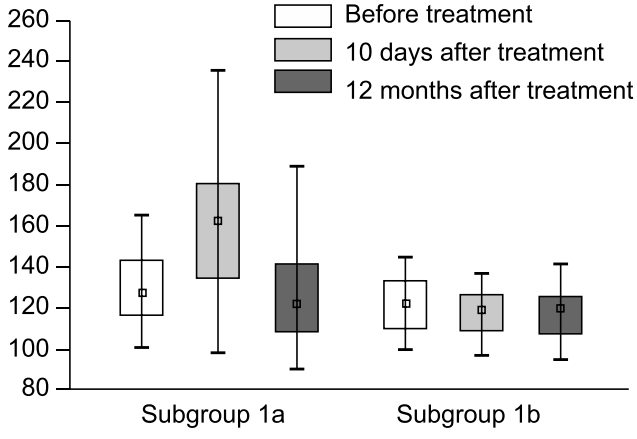


**Fig. 3.** The concentration of IFN-γ in women with CIN I associated with papillomavirus infection, pg/ml.

In the subgroup 2 of patients with CIN I (1b), who were under the case follow-up, the IL-18 level during the study did not change both after 10 days and 12 months, and corresponded to the initial indicator of 9.11±0.10 pg/ml (Fig. 2).

The reduced level of IL-18, which is significant in the formation of an immune response involving $CD8^+$ T-lymphocytes in the cervical mucus of patients with CIN I, is probably associated with the immunosuppressive effect of the HPV E6 protein. It is the E6 protein that binds to interleukin-18, which is the main inducer of interferon gamma, leading to a blockade of cellular cytotoxic immune reactions.

Attention was paid to a wide range of fluctuations in the level of IFN-γ in women who used Alloferonum. IFN-γ significantly increased in the patients of subgroup 1a from 127.32±2.60 to 159.65±4.83 pg/ml, respectively, 10 days after treatment in comparison with patients of subgroup 1b (Fig. 3).

Given the activating effect of IL-18 on the synthesis of IFN-γ and the antiviral immune response, an increase in its level 10 days after exposure to Allokin-alpha can be considered a positive aspect of therapy.

Previous studies have shown that TNF-α inhibits the proliferation of healthy epithelial cells of the cervix, and in the case of infection of the epithelium with HPV types 16 and 18, the same TNF-α already stimulates the proliferation of affected cells [13].

Insufficient production of TNF-α maintains long-term persistence of the virus and causes malignant transformation of epithelial cells, which in turn leads to the formation of a cervical intraepithelial lesion (Fig. 4).

Inhibition of apoptosis can occur as a result of a constant, long-term effect of various groups of cytokines on the caspase cascade. Changes in the cytokine profile in comparison with those of healthy women are presented in Table 4.

**Table 4.** Comparison of the indicators of the study groups (changes in the cytokine profile) with the indicators of the control group

| Indicator | Control group (*n*=43) | CIN I, before treatment | After 10 days | | After 12 months | | Significance level | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | subgr. 1a (*n*=28) | subgr. 1b (*n*=27) | subgr. 1a (*n*=28) | subgr. 1b (*n*=27) | $p_1$ | $p_2$ | $p_3$ | $p_4$ |
| IFN-γ, ng/ml | 89.44± 0.85 | 123.85± 1.66 | 159.65± 4.83* $p_4$ | 116.46± 1.66* $p_1$, $p_3$ | 127.23± 3.96* $p_4$ | 115.68± 1.80* $p_2$, $p_3$ | 0.0000 | 0.0974 | 0.0644 | 0.002 |
| IL-18, ng/ml | 8.12± 0.07 | 8.97± 0.12* | 12.61± 0.35* $p_4$ | 9.12± 0.10* $p_1$, $p_3$ | 11.58± 0.21 $p_4$ | 9.10± 0.10* $p_2$, $p_3$ | 0.0000 | 0.0000 | 0.2897 | 0.001 |
| TNF-α, ng/ml | 2.66± 0.05 | 2.76± 0.05 | 2.72± 0.08* $p_4$ | 2.79± 0.06* $p_1$, $p_3$ | 2.73± 0.08* $p_4$ | 2.80± 0.06* $p_2$, $p_3$ | 0.5135 | 0.5190 | 0.6000 | 0.001 |

The symbol * indicates values that have significant differences with the control group (Mann–Whitney test, $p$ <0.05); $p_1$ — significant difference in the parameters of subgroup 1a compared with subgroup 1b after 10 days ($p$ <0.05); $p_2$ — significant difference in the indices of subgroup 1a compared with subgroup 1b after 12 months ($p$ <0.05); $p_3$ — significant difference in the indices of subgroup 1a compared with values before treatment ($p$ <0.05); $p_4$ — significant difference in the indices of subgroup 1b with values before treatment ($p$ <0.05).

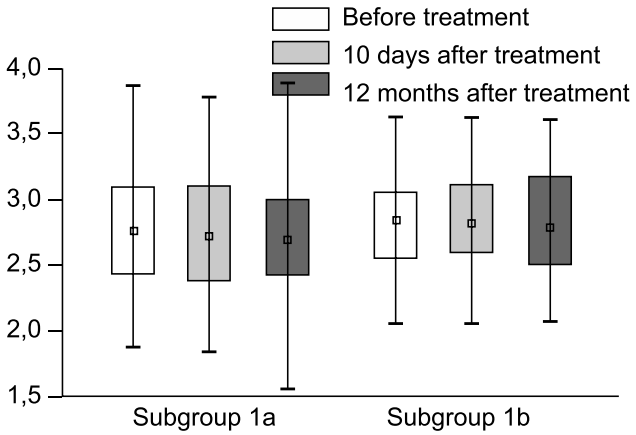


**Fig. 4.** The concentration of TNF-α in the cervical mucus of the studied groups of women with CIN I, pg/ml.

When analyzing the changes in viral load together with the cytokine profile in the study groups and considering the possibility of the effect of the virus on the processes of apoptosis inside the cell, 3 and 6 months after the treatment, we determined the levels of caspase 3 and caspase 9 and obtained the following results (Table 5).

Analysis of the caspase 3 and caspase 9 values in the subgroups after 3 and 6 months revealed that all indicators differed significantly between the subgroups and relative to the values before treatment. The values of caspase 3 and caspase 9 in subgroup 1b were lower than their values for the same period in subgroup 1a. It should be noted that the dynamics of the decrease in the caspases 3 and 9 levels in group 1b slowed down by the 6$^{th}$ month of follow-up. If, the level of caspase 3 in group 1b decreased by 42% from the initial level compared to 22% in the group 1a after 3 months, then the decrease was 14% in the group 1b and 12% in the group 1a after another three months. The situation was similar with caspase 9, but less significant. Apparently, for more intensive treatment, a repeated course of Allokin-alpha is required three to four months after the start of treatment (Table 6).

The E6 protein mentioned above, along with other E-group proteins, is responsible for virus replication and host cell transformation. Moreover, it is a protooncogene [17]. The use of Allokin-alpha as part of the complex therapy for CC led to an 8-fold decrease in the concentration of E6 protein in the tumor tissue. In the group of patients who received only standard cytostatic therapy, the concentration of E6 protein in the tumor decreased by only 2.5 times [18]. Perhaps, it is the ability of Allokin-alpha to reduce the concentration of the E6 protein and block its action that explains the absence of CIN progression, a high percentage of normalization of the cervical mucosa with incomplete elimination of HPV in group 1a [19, 20].

Since, during the formation of CIN I, the virus penetrates the epithelial cells of the basal layer and is in an episomal form, this allows HPV to remain unrecognized by the immune system. According to some researchers, mild cervical neoplasia indirectly indicates the activity of the immune system, and it is possible that the established level of caspases is sufficient for the lysis of atypical cells. However, under the influence of antiviral treatment in patients, the process of lysis of virus-infected cells of the stratified squamous epithelium occurred more intensively, since under the influence of Alloferonum, T-lymphocytes were activated due to an increase in the availability of cells affected by the virus, as evidenced by a more dynamic decrease in the levels of caspase 3 and caspase 9 in subgroup 1a.

## CONCLUSION

The results obtained confirm the changes in the immune status that occur under the influence of HPV. The process of cell self-destruction is multistage and polycascade, involving several components of the cellular vital activity.

The revealed imbalance of pro-inflammatory and anti-inflammatory cytokines in favor of the latter is an important factor supporting the persistence of HPV in the cervical

**Table 5.** Comparison of the caspase 3 and caspase 9 levels at 3 and 6 months after treatment

| Indicator | Control group (*n*=43) | CIN I before treatment | After 3 months | | After 6 months | | Significance level | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | subgr. 1a (*n*=28) | subgr. 1b (*n*=27) | subgr. 1a (*n*=28) | subgr. 1b (*n*=27) | $p_1$ | $p_2$ | $p_3$ | $p_4$ |
| Caspase 3, ng/ml | 0.179± 0.02 | 2.772± 0.03* | 1.613± 0.04* $p_4$ | 2.164± 0.05* $p_1$, $p_3$ | 1.391± 0.04* $p_4$ | 1.904± 0.05* $p_2$, $p_3$ | 0.0308 | 0.0029 | 0.001 | 0.002 |
| Caspase 9, ng/ml | 0.213± 0.03 | 2.311± 0.05* | 1.474± 0.05* $p_4$ | 1.904± 0.05* $p_1$, $p_3$ | 1.029± 0.05* $p_4$ | 1.673± 0.05* $p_2$, $p_3$ | 0.0028 | 0.001 | 0.003 | 0.001 |

Note: Statistical significance of the changes in caspase 3 and caspase 9 activity was assessed according to the Wilcoxon–Mann–Whitney criteria. The symbol * indicates values that have significant differences with the control group (Mann–Whitney test, $p$ <0.05); $p_1$ — significant difference in the parameters of subgroup 1a compared with subgroup 1b after 3 months ($p$ ≤0.05); $p_2$ — significant difference in the indices of subgroup 1a compared with subgroup 1b after 6 months ($p$ <0.05); $p_3$ — significant difference in the indices of subgroup 1a compared with values before treatment ($p$ ≤0.05); $p_4$ — significant differences in the indices of subgroup 1b compared with values before treatment ($p$ ≤0.05).

**Table 6.** Analysis of the cytological results and detectability of CIN I in the study groups after 12 months

| TBS category change* as a result of treatment** | CIN I, subgroup 1a (*n*=28) | CIN I, subgroup 1b (*n*=27) |
|---|---|---|
| CIN I, % | 27.90 | 44.19 |
| NILM, % | 72.09 | 39.53 |
| CIN II, % | 0.00 | 16.27 |

* Bethesda terminology system; ** according to liquid oncocytology; NILM — result negative for dysplasia or cancer.

epithelium in grade 1 CIN. An increase in the level of the cytokine IL-18 indicates a change toward cellular immunity, stimulation of IFN- and Fas-ligand-mediated apoptosis, which probably contributes to the elimination of HPV.

The protective mechanisms against the infection of healthy cells proceed with the participation of initiating caspase 9 and effector caspase 3. A decrease in the values of caspase 3 and caspase 9 is a favorable sign, since the intensity of apoptosis decreases in the absence of an HPV trigger effect on the cell.

During the study, the efficiency of treatment depending on the chosen approach was analyzed, which showed that the greatest elimination of HPV infection was recorded in patients who were treated with the antiviral drug Allokin-alpha.

For the fastest elimination of the pathogen, normalization of the colposcopic presentation, and complete recovery of patients, a repeated course with Allokin-alpha 3–4 months after the start of treatment can be recommended.

## ADDITIONAL INFO

**Author contribution.** All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

**Funding source.** This study was not supported by any external sources of funding.

**Competing interests.** The authors declares that there are no obvious and potential conflicts of interest associated with the publication of this article.

## REFERENCES


**1.** Bebneva TN, Muizhnek EL, Rogovskaya SI, et al. Cervical neoplasia: new approaches to pathogenesis-oriented treatment. *Doctor.Ru.* 2016;(3):9–14. (In Russ).

**2.** Svidinskaya EA, Dzhibladze TA, Zuev VM. Role of determination of molecular genetic markers in diagnostics and prognosis of cervical diseases course. *Tumours of female reproductive system.* 2010;(2):93–98. (In Russ).
doi: 10.17650/1994-4098-2010-0-2-93-98

**3.** Zur HH. Papillomaviruses causing cancer: evasion from host-cell control in carcinogenesis. *J Natl Cancer Inst.* 2000;92:690–698. doi: 10.1093/jnci/92.9.690

**4.** Fahey JV, Schaefer TM, Channon JY, Wira CR. Secretion of cytokines and chemokines by polarized human epithelial cells from the female reproductive tract. *Hum Reprod*. 2005;20(6):1439–1446. doi: 10.1093/humrep/deh806

**5.** Lieberman JA, Moscicki A-B, Sumerel JL, Ma Y, Scott ME. Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates. *Clin Vaccine Immunol*. 2008;15(1):49–54. doi: 10.1128/cvi.00216-07

**6.** Bergeron Ch, Ikenberg H, Sideri M, et al. Evaluation of p16/Ki-67 Dual-Stained Cytology for Managing Women With Abnormal Papanicolaou Cytology: PALMS Study Results. *Cancer Cytopathol*. 2015;123(6):373–381. doi: 10.1002/cncy.v123.11

**7.** Zima AP, Ryazantseva IV, Novitsky VV. The system of tumor necrosis factor and its receptors in immunopathogenesis of persistent viral infections. *Immunology*. 2007;(6):357–361. (In Russ).

**8.** Keppler D, Lin A, eds. *Cervical Cancer: Methods and Protocols*. NY: Humana Press; 2015. XIV. 413 p.
doi: 10.1007/978-1-4939-2013-6

**9.** Vinogradova OP, Artemova OI. Pathogenic aspects of apoptotic program change during HPV-associated pathologies of cervix. *Ural Medical Journal.* 2019;(15):100–106. (In Russ).
doi: 10.25694/URMJ.2019.15.21

**10.** Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*. 2014;(32):513–545.
doi: 10.1146/annurev-immunol-032713-120231

**11.** Vyazovaya AA, Kuyevda DA, Trofimova OB, et al. The identification of viruses of human papilloma of high carcinogenic risk and evaluation of physical status of viral DNA using technique of polymerase-chain reaction under affection of cervical epithelium. *Russian Clinical Laboratory Diagnostics.* 2013;(8):2426. (In Russ).

**12.** Polonskaya NYu, Yurasova IV. *Cytological examination of cervical smears. PAP test.* Moscow: GEOTAR-Media; 2018.168 p. (In Russ).

**13.** Trushina OI, Novikova EG, Shipulina OYu, Romanyuk TN. Viral load of HPV DNA as a prognostic factor of malignant progression of PVI. In: *V.I. Pokrovsky, ed. Molecular diagnostics — 2014: Proceedings of the VIII All-Russian Scientific and Practical Conference with international participation.* Moscow; 2014. (In Russ).

**14.** Abramovskikh OS, Dolgushina VF, Telesheva LF. Cytokines in neoplastic processes of the cervix associated with the human papilloma virus of high carcinogenic risk. *Russian journal of immunology*. 2011;5(1):79–81. (In Russ).

**15.** Konovalova NV, Khramenko NI, Velichko LN. Clinical eye condition and tissue immunity dynamics of patients with iridocyclitis under the influence of antiviral drug allokin-alpha. *Point of View. East–West*. 2017;(3):57–60. (In Russ).

**16.** Kutsenko II, Borovikov IO, Dekhtyarenko YuV, Bulgakova VP. Complex therapy of recurrent genital herpes in women. *RUDN Journal of Medicine*. 2012;(5):334–341. (In Russ).

**17.** Dueñas-González A, Lizano M, Candelaria M, et al. Epigenetics of cervical cancer. An overview and therapeutic perspectives. *Mol Cancer*. 2005;4:38. doi: 10.1186/1476-4598-4-38

**18.** Men'shenina AP, Moiseeva TI, Ushakova ND, et al. Plasmapheresis and nonspecific immunotherapy effects on treatment of patients with locally advanced forms of cervical cancer. *Zhurnal nauchnykh statei «Zdorov'e i obrazovanie v XXI veke».* 2016;18(7):50–53. (In Russ).

**19.** Epifanova OV, Vinogradova OP, Andreeva NA. Immune antiviral drug therapy in patients with HPV-associated cervical intraepithelial neoplasia grade I. *Obstetrics and Gynecology.* 2020;(3):174–180. (In Russ). doi: 10.18565/aig.2020.3.174-180

**20.** Khaitov R. *Immunology: structure and functions of the immune system: a textbook*. Moscow: GEOTAR-Media; 2013. P. 204–207. (In Russ).


## СПИСОК ЛИТЕРАТУРЫ


**1.** Бебнева Т.Н., Муйжнек Е.Л., Роговская С.И., и др. Патогенетическое лечение неопластических процессов шейки матки: новые подходы // Доктор.Ру. 2016. № 3(120). С. 9–14.

**2.** Свидинская Е.А., Джибладзе Т.А., Зуев В.М. Роль определения молекулярно-генетических маркеров в диагностике и прогнозировании течения заболеваний шейки матки // Опухоли женской репродуктивной системы. 2010. № 2. С. 93–98.
doi: 10.17650/1994-4098-2010-0-2-93-98

**3.** Zur H.H. Papillomaviruses causing cancer: evasion from host-cell control in carcinogenesis // J Natl Cancer Inst. 2000. Vol. 92. P. 690–698. doi: 10.1093/jnci/92.9.690

**4.** Fahey J.V., Schaefer T.M., Channon J.Y., Wira C.R. Secretion of cytokines and chemokines by polarized human epithelial cells from the female reproductive tract // Hum Reprod. 2005. Vol. 20, N 6. P. 1439–1446. doi: 10.1093/humrep/deh806

**5.** Lieberman J.A., Moscicki A.-B., Sumerel J.L., Ma Y., Scott M.E. Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates // Clin Vaccine Immunol. 2008. Vol. 15, N 1. P. 49–54. doi: 10.1128/cvi.00216-07

**6.** Bergeron Ch., Ikenberg H., Sideri M., et al. Evaluation of p16/Ki-67 Dual-Stained Cytology for Managing Women With Abnormal Papanicolaou Cytology: PALMS Study Results // Cancer Cytopathol. 2015. Vol. 123, N 6. P. 373–381.
doi: 10.1002/cncy.v123.11

**7.** Зима А.П., Рязанцева И.В., Новицкий В.В. Система фактора некроза опухоли α и его рецепторов в иммунопатогенезе персистентных вирусных инфекций // Иммунология. 2007. № 6. С. 357–361.

**8.** Keppler D., Lin A., eds. Cervical Cancer: Methods and Protocols. NY : Humana Press, 2015. Vol. XIV. 413 p.
doi: 10.1007/978-1-4939-2013-6
**9.** Виноградова О.П., Артемова О.И. Патогенетические аспекты изменения апоптотической программы при ВПЧ-ассоциированных патологиях шейки матки // Уральский медицинский журнал. 2019. № 15(183). С. 100–106.
doi: 10.25694/URMJ.2019.15.21
**10.** Schneider W.M., Chevillotte M.D., Rice C.M. Interferon-stimulated genes: a complex web of host defenses //Annu Rev Immunol. 2014. N 32. P. 513–545. doi: 10.1146/annurev-immunol-032713-120231
**11.** Вязовая А.А., Куевда Д.А., Трофимова О.Б., и др. Выявление вирусов папилломы человека высокого канцерогенного риска и оценка физического статуса вирусной ДНК методом ПЦР при поражении цервикального эпителия // Клиническая лабораторная диагностика. 2013. № 8. С. 24–26.
**12.** Полонская Н.Ю., Юрасова И.В. Цитологическое исследование цервикальных мазков. Пап-тест. Москва : ГЭОТАР-Медиа, 2018. 168 с.
**13.** Трушина О.И., Новикова Е.Г., Шипулина О.Ю., Романюк Т.Н. Вирусная нагрузка ДНК ВПЧ как прогностический фактор злокачественной прогрессии ПВИ. В кн.: Покровский В.И., ред. Молекулярная диагностика — 2014: сборник трудов VIII Всероссийской научно-практической конференции с международным участием. Москва, 2014. 207 с.
**14.** Абрамовских О.С., Долгушина В.Ф., Телешева Л.Ф. Цитокины при неопластических процессах шейки матки, ассоциированных с вирусом папилломы человека высокого канцерогенного риска // Российский иммунологический журнал. 2011. Т. 5, № 1. С. 79–81.
**15.** Коновалова Н.В., Храменко Н.И., Величко Л.Н. Клиническое состояние глаз и динамика тканевого иммунитета у больных иридоциклитом под влиянием противовирусного препарата аллокин-альфа // Точка зрения. Восток – Запад. 2017. № 3. С. 57–60.
**16.** Куценко И.И., Боровиков И.О., Дехтяренко Ю.В., Булгакова В.П. Комплексная терапия рецидивирующего генитального герпеса у женщин // Вестник РУДН. Серия: Медицина. 2012. № 5. С. 334–341.
**17.** Dueñas-González A., Lizano M., Candelaria M., et al. Epigenetics of cervical cancer. An overview and therapeutic perspectives // Mol Cancer. 2005. Vol. 4. P. 38. doi: 10.1186/1476-4598-4-38
**18.** Меньшенина А.П., Моисеева Т.И., Ушакова Н.Д., и др. Влияние плазмафереза и неспецифической иммунотерапии на результаты лечения больных местно-распространенными формами рака шейки матки // Журнал научных статей «Здоровье и образование в XXI веке». 2016. Т. 18, № 7. С. 50–53.
**19.** Епифанова О.В., Виноградова О.П., Андреева Н.А. Особенности консервативной иммунопротивовирусной терапии пациенток с ВПЧ-ассоциированными цервикальными интраэпителиальными неоплазиями I степени // Акушерство и гинекология. 2020. № 3. С. 174–180.
doi: 10.18565/aig.2020.3.174-180
**20.** Хаитов Р. Иммунология: структура и функции иммунной системы: учебное пособие. Москва : ГЭОТАР-Медиа, 2013. С. 204–207.

## AUTHORS INFO

***Ol'ga P. Vinogradova,** M.D., Dr. Sci. (Med.), professor;
address: 8A Stasov str., Penza, 440060, Russian Federation;
ORCID ID: http://orcid.org/0000-0002-9094-8772;
e-mail: o_vinogradova69@mail.ru

**Natal'ya A. Andreeva,** MD, Cand. Sci. (Med.), assistant professor;
ORCID ID: http://orcid.org/0000-0002-2207-7039;
e-mail: andreeva_77@list.ru

**Ol'ga V. Epifanova,** MD, assistant;
ORCID ID: http://orcid.org/0000-0002-3961-809X;
e-mail: epifanova.vrt@gmail.com

**Ol'ga I. Artemova,** MD, junior researcher;
ORCID ID: http:// orcid.org/0000-0002-4996-026X;
e-mail: artyomovaolg@gmail.com

## ОБ АВТОРАХ

***Виноградова Ольга Павловна,** д.м.н., профессор;
адрес: 440060, г. Пенза, ул. Стасова, 8А, Россия;
ORCID ID: http://orcid.org/0000-0002-9094-8772;
e-mail: o_vinogradova69@mail.ru

**Андреева Наталья Анатольевна,** к.м.н.;
ORCID ID: http://orcid.org/0000-0002-2207-7039;
e-mail: andreeva_77@list.ru

**Епифанова Ольга Викторовна,** ассистент;
ORCID ID: http://orcid.org/0000-0002-3961-809X;
e-mail: epifanova.vrt@gmail.com

**Артёмова Ольга Игоревна,** младший научный сотрудник;
ORCID ID: http:// orcid.org/0000-0002-4996-026X;
e-mail: artyomovaolg@gmail.com

* Автор, ответственный за переписку / Corresponding author