Medical center of the President's Affairs Administration

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Director of the Medical center

Hospital of the President's Affairs

Administration of the Republic of Kazakhstan

R.K. Albayev

«__» _____ 2024

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RESEARCH REPORT

ON THE TOPIC:

«THE DEVELOPMENT OF INNOVATIVE TECHNOLOGIES INCREASING DIAGNOSIS AND TREATMENT EFFICIENCY OF THE CERVIX BACKGROUND AND PRECANCEROUS DISEASES ASSOCIATED WITH HPV»

(final)

Program Code O.1088

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ABSTRACT

Report 89 pages, 1 book, 10 figures, 9 tables, 54 references, 6 appendices.

CERVICAL CANCER, CERVICAL PREMALIGNANT LESIONS, HUMAN PAPILLOMAVIRUS, PHOTODYNAMIC THERAPY, CERVICAL INTRAEPITHELIAL NEOPLASIA

The subject of the study is the diagnosis and treatment of background and precancerous diseases of the cervix associated with human papillomavirus (HPV).

The aim of the work is to develop innovative technologies that enhance the effectiveness of diagnosis and treatment of background and precancerous conditions of the cervix associated with HPV.

Scientific novelty: For the first time in the Republic of Kazakhstan, the method of photodynamic therapy (PDT) was used and optimized with a combination of local and systemic laser exposure, as well as the testing of the hypothesis regarding the possible cumulative effect of laser technology with photosensitization and fluorescent diagnostics using a photosensitizer for diagnosing and treating cervical dysplasia associated with HPV.

In our study, we determined that married women and those with a history of abortions were most prone to developing cervical dysplasia (CIN), and could be included in the risk group for CIN ($p=0.02$ and $p=0.03$, respectively). Overall, among 150 patients, the most frequently detected HPV genotype was type 16 (44%), followed by types 31 (13.2%), 52 (10.5%), and 58 (10.5%). The prevalence of HPV types 52 and 58 significantly differed depending on the severity of dysplasia, being more common in severe cases ($p=0.01$ and $p=0.03$, respectively).

Our study showed that fluorescent diagnostics indicated brighter fluorescence in the severe dysplasia group compared to the moderate dysplasia group, suggesting that the greater the number of atypical cells, the brighter the fluorescence.

Assessment of complete regression of the pathological process at 12 months post-PDT showed that the complete recovery rate in the moderate dysplasia group was 96.1%, while the recovery rate for the severe dysplasia group was 92.3%. Our therapy demonstrated better results compared to international data, where the recovery rate for severe dysplasia ranged from 66.7% to 92.73%, while for moderate dysplasia it varied from 57.1% to 83.3%. However, statistical analysis revealed differences in treatment outcomes between the severe and moderate CIN groups, indicating that the presence of more than two identified STIs prior to PDT could be a factor reducing treatment effectiveness ($p=0.003$).

We also demonstrated that PDT stimulates the release and expression of various pro-inflammatory mediators from the area subjected to PDT, activating the immune response against the virus and promoting the healing of the cervix. In particular, levels of IL-6 and TNF-α on the

5th day after PDT were significantly higher in the severe dysplasia group compared to the moderate dysplasia group ($p<0.001$).

As part of the study, the genetic variability of the HLA system (HLA-DQB1; HLA-DQA2; TAP2; HLA-C) in patients with cervical dysplasia based on HPV types was analyzed for the first time in the world. Polymorphic variants rs62619945 and rs200904145 in the HLA-DQA2 gene showed significant influence on carriers of HPV type 18 compared to carriers of other HPV variants ($p=0.01$ and $p=0.008$, respectively), while the prevalence of polymorphic variants rs9276437, rs200904145, rs34730447, and rs201291459 in the HLA-DQA2 gene was highest among HPV type 51 carriers compared to carriers of other HPV variants ($p=0.03$, $p=0.004$, $p=0.03$, and $p=0.004$, respectively). Thus, our results indicate that alleles of the HLA-DQA2 gene play a role in the persistence of HPV types 18 and 51, as well as in the immune response to the virus in Kazakh women.

All of the above results formed the basis for the algorithm we developed for managing patients with oncogenic types of HPV for practical application. The proposed approach within medical services includes the following stages: screening, preventive rehabilitation, managed oncogenesis, sub-managed oncogenesis, and unmanaged oncogenesis. The approach suggested in the algorithm for using PDT in cases of HPV persistence is based on the collection and analysis of data reflecting individual patterns of oncogenesis. The algorithm accounts for the sequence of biological processes, starting from immune response instability with HPV and ending with irreversible changes in cervical cancer. This approach allows for management of the duration of the "pre-disease" period. Furthermore, understanding the causes of abnormal immune responses related to genetic risk factors may help develop new strategies for managing HPV infection and associated diseases aimed at improving clinical effectiveness in combined strategies against cervical cancer. Such implementation into medical practice may contribute to reducing the burden of HPV in the medium term through early intervention at the level of risk factors.

Degree of implementation: The new technology is a scientific product for practical application and is significant for future research directions and shifting the focus of clinical practice.

Area of application: personalized medicine, population medicine, clinical medicine, epidemiology, public health.

TABLE OF CONTENT

TERMS AND DEFINITIONS

In this research report, the following terms are used with their respective definitions:

Human Papillomavirus (HPV)	a group of highly prevalent and genetically diverse DNA-containing viruses that affect the epithelium of skin and mucous membranes. Human papillomavirus belongs to the papillomavirus family. Depending on their oncogenic potential, high-risk (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and low-risk (types 6, 11, 42, 43, 44) oncogenic risk types are distinguished.
Genotype	the combination of alleles of a gene or locus in a specific organism.
Genotyping	the process of determining a genotype.
Personalized Medicine	a set of methods for the prevention of pathological conditions, diagnosis, and treatment based on individual patient characteristics.
Genome-Wide Association Study (GWAS)	a field of biological (usually biomedical) research related to the study of associations between genomic variants and phenotypic traits.
Gene Polymorphisms (Genetic Polymorphism)	the diversity of gene alleles (different forms of the same gene), which leads to the diversity of intra-species traits.
Cervical Cancer	a malignant tumor originating from the mucous membrane of the cervix (ectocervix or endocervix).
Pharmacogenetics	a branch of medical genetics and clinical pharmacology that studies the hereditary features of drug effects' variability and predicts the effectiveness and safety of drug use in patients.
Pharmacogenomics	a branch of pharmaceuticals and pharmacology that studies genetic variations in each person's response to medication.
Phenotype	a set of characteristics inherent to an individual at a certain stage of development.
Photodynamic Therapy and Fluorescence Diagnostics	a systemic and local-regional method for diagnosing and treating malignant neoplasms. The method is based on the selective accumulation of specific photosensitizing agents (photosensitizers) in pathological tissues, including tumor

	tissue, capable of causing photochemical reactions in biological tissues after exposure to light of a certain wavelength.
Photolon	a third-generation photosensitizer for photodynamic diagnosis and therapy. The photosensitizer Photolon, based on chlorin E6, is currently one of the five approved agents for clinical use in photodynamic diagnosis and therapy of malignant neoplasms.
Photosensitizer	medicinal preparations capable of accumulating in affected tissues in higher quantities compared to healthy tissues. When the examined organ is irradiated with low-intensity laser radiation, the affected areas are visually detected through fluorescence or using laser-fiber spectral analysis.

LIST OF ABBREVIATIONS AND DESIGNATIONS

In this research report, the following abbreviations and designations are used:

The Hospital	Hospital of the President's Affairs Administration of the Republic of Kazakhstan
IVLB	Intravenous Laser Blood Irradiation
WHO	World Health Organization
HPV	Human Papillomavirus
HR-HPV	High-Risk Human Papillomavirus
CI	Clinical Trials
R&D	Research and Development
CBC	Complete Blood Count
CC	Cervical Cancer
SPDT ILBI	Systemic Photodynamic Therapy during Intravenous Laser Blood Irradiation
USA	United States of America
FD	Fluorescence Diagnostics
FDI	Fluorescence Diagnostic Investigation
PDT	Photodynamic Therapy
TNF	Tumor Necrosis Factor
PS	Photosensitizer
CIN	Cervical Intraepithelial Neoplasia
SHEQAC MSHE RK	Science and Higher Education Quality Assurance Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan

INTRODUCTION

Among all cases of cancer worldwide, approximately 15% to 20% are associated with viral infections, making oncogenic viruses significant risk factors for cancer development [1]. One of the most well-known infectious oncogenic agents is the human papillomavirus (HPV), responsible for 31.1% of all cancer cases caused by infectious diseases and for 99.7% of cervical cancer cases in women [1, 2]. Although 90% of HPV infections are temporary and are cleared by the immune system within 12–24 months after exposure, some persistent strains of HPV can lead to uncontrolled proliferation of infected cells, resulting in precancerous or tumor changes in the host's body [3].

Cervical cancer ranks fourth among the most common cancers in women, with over 80% of cases linked to the presence of oncogenic HPV that persists in the body. In 2020, approximately 604,127 cases of cervical cancer were reported globally, with 341,680 resulting in death [4]. Cervical cancer accounts for 7.7% of cancer mortality among women [5]. The five-year survival rate for women with cervical cancer in 2020 varied by country, ranging from 37% to 77% [5].

Overall, the mortality rate from cervical cancer in developing countries is 18 times higher than in more affluent Western countries [5]. This disparity is attributed to a lack of public awareness, socioeconomic factors, absence of HPV vaccination, and limited access to screening programs, along with delays or inadequate treatment [6].

The reduction of cervical cancer incidence is linked to the implementation of the WHO-initiated triple intervention strategy in 2018 aimed at eliminating cervical cancer as a public health issue in the 21st century: vaccinating 90% of girls under the age of 15, conducting two rounds of integrated HPV-based screening for 70% of women aged 35 to 45, and treating at least 90% of all precancerous lesions identified during screening [5].

In the Republic of Kazakhstan, cervical cancer is the second most prevalent cancer among women and the second most frequent disease among women aged 15 to 44 [7, 8]. According to the mandatory cancer screening program in Kazakhstan, women aged 30 to 70 are screened every four years. In 2021, 757,454 women in this age group were screened, with precancerous conditions identified in 0.99% (7,498) of women, and cervical cancer diagnosed in 0.04% (319) of them. The cervical cancer mortality rate in Kazakhstan was 6.0 per 100,000 women in 2021 [4, 9], sharply contrasting with mortality rates in Western Asia or Western Europe, which were 1.9 and 2.2 per 100,000 women, respectively [3, 4].

Cervical cancer is a visually accessible form of tumor, and with the use of available and informative diagnostic methods, such as the Pap smear, colposcopy, and testing for high-risk HPV DNA, precancerous changes can be detected early, allowing for timely treatment. This can significantly reduce the risk of developing cervical cancer [3].

In line to prevent cervical cancer, the treatment of precancerous and background conditions should be comprehensive and effective. At the same time, it is crucial to preserve the anatomical and functional integrity of the cervix, which is important for the health of a woman's reproductive system.

The aim of the project is to develop innovative technologies that enhance the effectiveness of diagnosing and treating HPV-associated background and precancerous conditions of the cervix.

MAIN PART OF THE REPORT ON SCIENTIFIC RESEARCH WORK

1 Persistent HPV Infection as the Primary Cause of Precancerous Cervical Conditions

With the popularization of cervical cancer screening in recent years, HPV infection is increasingly being detected. Persistent HPV infection has been identified as the primary cause of cervical dysplasia, a precursor to invasive cervical cancer. There are more than 120 different types of HPV, which their risk of causing cervical cancer can classify. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered high-risk for cancer development, while types 6, 11, 42, 43, and 44 are classified as low-risk and are associated with most benign lesions of the anogenital area, such as genital warts [10, 11].

Most cases of cervical cancer worldwide are linked to the persistence of oncogenic HPV, particularly types 16 and 18, which are widely distributed globally [10, 12]. High-risk oncogenic HPV types can lead to the development of precancerous cervical dysplasia through the integration of HPV oncogenes into the host genome [13]. HPV infects the basal cells of the squamous epithelium of the cervix. Once inside the host cell, HPV DNA replicates as the basal cells differentiate and progress toward the epithelial surface [14]. The expression of HPV genes becomes independent of the differentiation status of the infected epithelial cells, and the uncontrolled expression of the viral genome leads to a significant increase in the expression of two HPV oncoproteins (E6 and E7) [14]. This results in the loss of normal control over the epithelial cell cycle, and cells begin to exhibit morphological features of basaloid-type cells in the upper half of the cervical epithelium [14]. These features can be observed during colposcopy after the application of acetic acid to the cervix.

Unlike some other viral infections, HPV evades activation of the innate immune system due to the absence of viremia or cell destruction following the initial infection in the cervix [14]. The explanation for eliminating HPV is based on specific immunological responses that require both cellular and competent humoral responses [15]. Changes in the mechanisms of the cellular immune response are responsible for the inability to clear HPV. On the other hand, it has been shown that immune tolerance promotes viral persistence and cancer progression [16]. Viral proteins inactivate two major tumor suppressor proteins (p53 and retinoblastoma protein (pRb)), leading to disruptions in DNA repair mechanisms and rapid cell proliferation [16]. This initiates a mechanism of sequential carcinogenesis with specific stages and progression of pathological processes in the cervix.

The results of cervical dysplasia screening are best interpreted using the unified and well-established Bethesda system [17]. This system includes the category of squamous intraepithelial lesions (SIL), covering the spectrum of squamous changes from low-grade intraepithelial lesions

(LSIL) (traditionally classified as cervical intraepithelial neoplasia I (CIN I) or "mild dysplasia") to high-grade squamous intraepithelial lesions (HSIL) (traditionally classified as CIN II/CIN III or "moderate dysplasia") and ultimately invasive squamous cell carcinoma [17]. Thus, SIL is closely linked to the early detection or prevention of cervical cancer [17]. There is evidence that normalization of the immune response at the level of low-grade dysplasia can halt and reverse carcinogenesis [13].

With an 80% prevalence of HPV globally, persistent infection develops in no more than 10% of infected individuals, while the remaining 70% maintain a transient infection. However, merely having a positive high-risk HPV status is insufficient for the development of cervical cancer. The onset of the disease requires interaction between poorly understood genetic and environmental factors [15, 16].

1.1 Photodynamic Therapy for Treating HPV-Associated Cervical Dysplasia

Traditional treatment methods for cervical dysplasia and HPV infection, such as radiation therapy, chemotherapy, cryotherapy, and surgical excision using laser or electrosurgical procedures, are invasive. These methods can lead to various side effects and complications, including bleeding, cervical stenosis, and serious issues in subsequent pregnancies, such as miscarriages, preterm births, and decreased fertility [18]. Additionally, the use of conventional treatments may result in multiple drug resistance, leading to ineffective treatment and disease recurrence. In many cases, HPV continues to persist, facilitating the progression of carcinogenesis [19]. Given that HPV-positive dysplasia is common among women of reproductive age and that the likelihood of reinfection remains high even after treatment, it is essential to develop effective strategies that minimise the risk of residual disease, malignancy, and reinfection while also preserving the patient's fertility. PDT stands out as a promising and highly selective therapeutic method in this context [19, 20]. To enhance the effectiveness of treatment for HPV-associated cervical conditions, a combined approach utilizing laser technologies has been proposed, involving the use of local PDT in conjunction with systemic photodynamic intravenous laser blood irradiation (ILBR) [21].

The mechanism of action of intravenous laser blood irradiation is based on its photobiological effects. In tissues saturated with light quanta, physical and chemical rearrangements of protein polymers occur, leading to changes in enzyme activity and the structural-functional properties of cell membranes. As a result of SPLBI, erythrocyte membrane permeability and deformability increase, aggregation capacity decreases, and the level of ATP 2,3-DPG rises [22, 23]. Consequently, the oxygen-transport function of the blood improves. In leukocytes, the activity of membrane receptors increases, DNA synthesis is activated, phagocytic properties are enhanced, and the secretion of bactericidal cationic proteins, rheological and

growth-stimulating factors, and interleukins is boosted. This also activates DNA repair enzyme systems and alters the reactivity of immunocompetent cells [24].

In platelets, the structure of membranes is modified, stimulating rheological factors, while plasma shows increased bactericidal, antioxidant, and proteolytic properties, as well as enhanced complement activity, lysozyme, natural and immune antibodies, and changes in coagulation and anticoagulation properties, with a significant decrease in lipid peroxidation products [19, 22]. These effects suggest a positive systemic immunological response, leading to prolonged virus elimination.

Fluorescent monitoring of the accumulation of photosensitizer in the lesion and its "burn-out" during laser irradiation is a necessary component of PDT, allowing for the standardization and optimization of treatment conditions.

1.2 PDT Mechanism of Action

Activation of the photosensitizer (PS) by light of the appropriate wavelength leads to the transition of the PS from its ground electronic state (S0) to one of its excited states (Sn) [37]. In biological environments, the excited PS then returns to its lowest excited state (S1) through internal conversion. From here, the photosensitizer can return to its ground state via heat dissipation, fluorescence emission, or conversion through intersystem crossing (ISC) to its lowest triplet state [37, 38]. This long-lived triplet state can lose energy through phosphorescence emission or energy transfer to the surrounding environment via collisional energy transfer. In the presence of molecular oxygen, the triplet oxygen ($^3O_2$) is converted to highly reactive singlet oxygen ($^1O_2$), which in turn leads to the formation of reactive oxygen species (ROS) (Figure 1), ultimately causing damage to vital cellular targets nearby [37, 38].

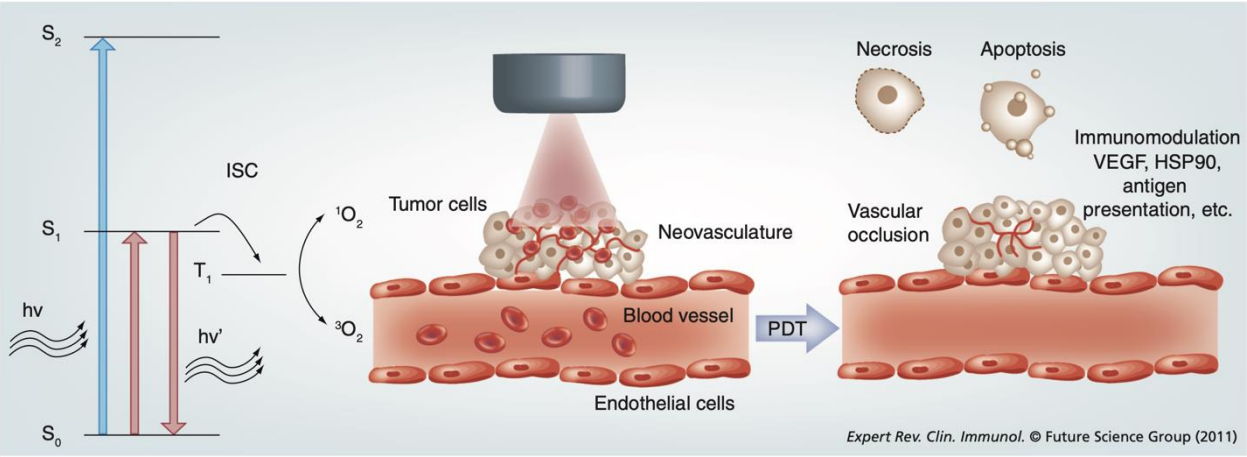


Figure 1 – Antitumor Mechanisms of Photodynamic Therapy [24].

The Jablonski diagram illustrates the absorption of light by the photosensitizer from its ground state, leading to the formation of a short-lived excited singlet state. This state can lose energy through fluorescence, internal conversion to heat, or transition to a long-lived triplet state of the photosensitizer, which is capable of undergoing photochemistry. This photochemistry subsequently results in the local generation of reactive oxygen species (ROS), which are cytotoxic to tumor and endothelial cells.

Key Terms: HSP: Heat shock proteins; hv: Light; ISC: Intersystem crossing; PDT: Photodynamic therapy; S0: Ground state; S1: First excited singlet state; S2: Second excited singlet state; T1: Triplet excited state.

It is important to note that only the simultaneous presence of all three components in PDT will lead to a significant photodynamic effect. Thus, even with less selective accumulation of the photosensitizer (PS) in the target tissue, the selectivity of PDT can be adjusted based on the presence or absence of one of the other two components.

1.3 Fluorescent Diagnosis of Precancerous Changes in the Cervix

The sensitivity of the method should be high enough to detect early-stage cervical dysplasia (LSIL). However, the procedure must also be simple, painless, and practical for implementation during routine examinations or screening programs. One of the most promising techniques for early diagnosis is known as optical biopsy. The term "optical biopsy" refers to any methodology that uses the interaction of light with tissue to obtain information about tissue morphology without the need for excision [39].

Precancerous and cancerous tissues differ from healthy tissues in their morphology and cell growth rates, leading to changes in optical characteristics. Most optical methods used in diagnostics are based on various types of spectroscopy, such as fluorescent, near infrared, and diffuse reflectance spectroscopy; however, the most widely used methods in clinical practice are those based on fluorescent phenomena. The adoption and applicability of these methods in clinical practice are determined by their diagnostic effectiveness, simplicity, and relatively low cost. Additionally, optical biopsy is non-invasive and can be repeated multiple times. Another advantage is that the contrast between healthy and pathological tissue can be enhanced using exogenous fluorescent agents or their precursors.

When entering the bloodstream, the photosensitizer (PS) binds to serum proteins, forming complex compounds [40]. These PS-protein complexes are absorbed by endothelial cells in blood capillaries, after which they bind to the vessel adventitia and enter the extracellular matrix, subsequently accumulating and becoming retained in pathologically altered cells. The mechanism of action involves exciting the molecule with short-wavelength light, such as ultraviolet light, causing it to emit a quantum of light with a longer wavelength when transitioning from the excited

state to the ground state [38]. Due to the high selectivity of the PS for precancerous cells, exciting it with blue light yields a distinct red fluorescent contrast between the precancerous tissue and the surrounding healthy tissue.

Fluorescent diagnostic methods for early forms of cancer have extremely high sensitivity, reaching up to 90%, but lower specificity. Because the penetration depth of the excitation radiation is limited, these methods can detect pathological foci located in the superficial layers of mucous membranes of hollow organs. The source of diagnostic information comes from the visual assessment of fluorescent images or the quantitative analysis of laser-induced fluorescence spectra measured in vivo at the tissue surface (the method of local fluorescent spectroscopy, LFS) [40]. When observing fluorescent images, pathological foci are identified by their abnormal fluorescence intensity against the background of healthy tissue fluorescence in the green spectral region. The verification of fluorescent images during LFS enhances the predictive value of fluorescent diagnostic methods.

The application of photodynamic (PD) methods in the comprehensive diagnosis of precancerous changes in the cervix enhances diagnostic efficiency by identifying the localization and size of lesions, contributing to more complete visualization for subsequent treatment [40]. The primary parameter for the reliability of this diagnostic method is the histological confirmation of the dysplastic status of fluorescent foci. Furthermore, there is a correlation between the degree of tissue dysplasia and fluorescence intensity. Fluorescent visualization can facilitate the detection of subclinical lesions.

Numerous randomized clinical trials have been conducted to determine the diagnostic value of fluorescent colposcopy compared to standard colposcopy, with the collection of biopsy material from tissues exhibiting clear fluorescent signals [28-30]. These studies assessed accuracy, sensitivity, specificity, and positive and negative predictive values [28, 29]. The results demonstrated a high potential for fluorescent colposcopy as a method for secondary screening of cervical pathology [30]. The high selectivity for tumours and low toxicity to healthy tissues make photosensitizer-based diagnostics a promising tool for the non-invasive identification and assessment of the severity (LSIL or HSIL) of cervical dysplasia.

1.4 Immune Response to PDT

An ideal method for treating cervical dysplasia should lead to local tumor regression and eradication, while also inducing a systemic antitumor immune response capable of effectively destroying HPV-infected epithelial cells without harming normal tissues. Photodynamic therapy (PDT) may meet these expectations, as it induces acute inflammation and recruits immune cells to target distant tumors [31].

Activation of the innate immune response is crucial for the subsequent induction of adaptive immunity [32]. The innate immune response consists of all protective mechanisms that lack immunological memory [32]. Thus, a defining characteristic of innate responses is their consistency, regardless of how frequently an antigen is encountered. The innate immune response involves various immune cells that participate in the first line of defense, and PDT has proven effective in engaging these cells in the inflammatory reactions of the body to dysplasia [32, 33].

Therefore, PDT initiates a powerful acute inflammatory response by activating the complement system, leading to a significant accumulation of neutrophils and other inflammatory cells at the treatment site, which aids in attacking atypical cells [33]. Complement fixation marks these cells as targets for destruction by the innate immune system. Complement not only acts as a direct mediator of inflammation but also stimulates cells to release secondary inflammatory mediators, including cytokines IL-1β, TNF-α, and IL-6 [34]. These factors contribute to the influx of innate immune cells into the tumor to attack and eliminate dying cancer cells. Numerous studies have highlighted the importance of PDT-induced inflammation in enhancing antitumor immunity.

After PDT, neutrophils also migrate to draining lymph nodes (DLNs) via high endothelial venules (HEV) through pathways mediated by IL-17, IL-1β, and MIP2 [33]. This infiltration of neutrophils into DLNs is transient and resolves within 24 hours post-PDT. The local and systemic neutrophilia induced by PDT enhances its effectiveness by destroying tumor tissue and activating antitumor CD8+ T cells [34, 35]. Several studies have shown that blocking the influx of neutrophils into tumors and DLNs reduces the effectiveness of PDT and decreases the number of activated antitumor CD8+ T cells [35].

1.5 Variations in the Variability of HLA-DQA2 Gene Sequences in HPV Persistence Among Kazakh Women

Susceptibility to HPV is influenced by the immune response. A persistently inadequate immune response allows the virus to initiate and sustain oncogenesis in the cervix. The cytological picture of cervical intraepithelial neoplasia, which develops asymptomatically, differs from the potential subsequent cancer, characterized by a high natural regression rate, presenting an opportunity to seek effective solutions for restoring autoimmune regulation.

The immune response is highly complex. In the early stages, interconnected processes of virus recognition, clearance, inflammation, and cell death are governed by the innate immune system. This task is performed by neutrophils, monocytes/macrophages, and dendritic cells through specialized pattern-recognition receptors. The adaptive immune response is more diverse and encompasses specialized immune reactions. Successful collaboration between the two types of immunity during transient HPV infection can clear the virus from a woman's body within up to 15 months.

If this process fails due to developing cellular changes, a complex and uncontrolled cell division process begins. These cellular changes are accompanied by the initiation of mechanisms for the joint synthesis of the viral and host cell genetic material (DNA), resulting in the integration of the virus into the host's genotype, making it indistinguishable from the immune defense. Consequently, the processes of proliferation and inhibition of apoptosis begin to progress, leading to cancer progression: HPV persistence – LSIL – HSIL – cervical malignancy [16].

It is known that the major histocompatibility complex (HLA), whose molecules are encoded by a dense cluster of genes located at chromosomal locus 6.21.3, plays a crucial role in ensuring the effectiveness of both innate and adaptive immune responses. This locus is highly polymorphic, and variations in the sequences of HLA proteins provide host susceptibility or resistance to many diseases [36]. Somatic mutation profiles are associated with HLA class I and class II alleles. HLA class I and class II are vital for binding and eliminating intracellular and extracellular foreign antigens.

The genetic variants underlying human susceptibility to high-risk HPV are still poorly understood [37]. Understanding the immunogenetic influence allows for the identification of molecular mechanisms that promote HPV persistence, provides a personalized approach for patients with a genetic risk of uncontrolled immune responses, and helps identify molecular defects as targets for developing effective strategies to manage HPV progression [38].

The most consistently identified allelic variation conferring protection against virus-induced cancers is located at locus 6.21.3 within the major histocompatibility complex (HLA) region, as confirmed in several populations of European and Asian descent [39]. The function of HLA system genes is to present the host with a cellular mechanism for immune control of the virus [40]. Primarily, this involves binding, delivering, and loading pathogen antigens onto the surface of antigen-presenting cells (T-lymphocytes, dendritic cells, macrophages) to ensure their accessibility to the antigen. The cytotoxic activity of T cells against infected cells plays a critical role in the adaptive immune response.

While HLA class I (HLA-DQB1, HLA-C, TAP2) mainly provides the early immune response, HLA class II (HLA-DQA2) is involved in long-term immunity, including antibody production and immunological memory. HLA-DQB1 and HLA-DQA2 play important roles in eliminating intracellular and extracellular foreign antigens, presenting two proteins that bind peptide fragments of the viral antigen macromolecule and form foreign antigens that stimulate the immune response [16, 38]. The HLA-C gene protein presents small peptides from within the cell and assists the TAP2 gene protein in transporting the viral antigen to the surface of CD8+ T cells, allowing the body to monitor for intracellular infections and mutations [16].

Different HLA alleles, by binding to various peptide fragments of the viral antigen macromolecule, form a diverse array of foreign antigens, which are then transported to the surface of antigen-presenting cells to interact with CD4+ and CD8+ T lymphocytes [41]. In this process, subtle differences in amino acid sequences are crucial for antigen elimination.

Polymorphisms in the HLA-DQB1 gene determine the specificity of antigen peptide binding. Associations have been recorded between HLA-DQB1 alleles and HPV types in cervical cancer, linked to weaker binding with HPV peptides compared to other alleles, leading to a more attenuated immune response [42]. Additionally, there is information regarding the influence of polymorphisms in the regulatory region of HLA-DQB1 on the expression of HLA molecules [43].

Thus, immunogenetic variability is an important reason for viral persistence, and identifying significant HLA genotypes enhances the interpretation of immunogenetic risks. In our study, 24 polymorphisms were identified (HLA-DQB1 -5; HLA-DQA2 – 11; TAP2 -4; HLA-C -4) that are involved in HPV persistence [41].

2 Materials and Methods

2.1 Conducting a Retrospective Cross-Sectional Comparative Analysis

The study was a retrospective cross-sectional comparative analysis of data from screening for early detection of cervical cancer (CC), conducted over an interval of 7 years. The study population consisted of Kazakh women over the age of 18 who were under medical supervision at the Hospital.

The Hospital has a well-organized cohort of women over 18 years old, totaling more than 10,000 individuals. Since 2016, as part of mandatory preventive examinations, all women undergo gynecological check-ups, which include liquid cytological analysis of cervical smears stained using the Papanicolaou method. In the case of atypical results, PCR detection and quantitative determination of HPV DNA genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, which are of high oncogenic risk, are performed. If the test is positive, colposcopy is conducted. The Hospital ensures timely screening and has a notification system to track abnormal results and actively invite patients, prioritizing screening. The staff consists of highly qualified professionals at all stages of the screening process.

The study involved a retrospective analysis of PCR real-time and liquid cytology findings performed at the BMC UDP laboratory during the periods from January 1, 2016, to December 31, 2016, and from January 1, 2022, to December 31, 2022. The results examined were obtained from Kazakh women aged 18 and older who presented at the Hospital for scheduled cervical cancer screening. A separate group included results from women with a known diagnosis of cervical cancer.

2.1.1 Data Sources

For the epidemiological analysis, the following sources were used:

- "Report on Malignant Diseases" (form No. 7) for the Republic of Kazakhstan for the years 2016 and 2022.
- Data from reports by the National Center for Healthy Lifestyle Promotion on the results of screening examinations for target population groups in Kazakhstan for 2016 and 2022.
- Data from the Hospital's laboratory service regarding the results of cytological examinations of cervical smears stained using the Papanicolaou method, as well as real-time PCR for detecting high-risk HPV DNA (12 genotypes).
- Data from the "Infomed" information database of the Hospital: demographic, clinical, and laboratory data.

2.2 Study Design and Patient Selection

A total of 200 Kazakh women participated in the case-control study. The recruitment of patients was carried out in the therapeutic department of the Hospital of the Medical Center of the

Office of the President of the Republic of Kazakhstan in the period from January 2023 to December 2024.

To optimize the study, some changes were made to the study protocol. Firstly, we increased the age threshold, because older patients have different types of HPV and more aggressive types of tumours and need a minimally invasive treatment method with a positive effect. Secondly, the main group included patients after surgery and conization, in whom these types of treatment did not show the desired effects. Thirdly, the interim examination interval included the 5th day after the PDT session (Protocol № 2 from 01.03.2023 г.) (Figure 2).

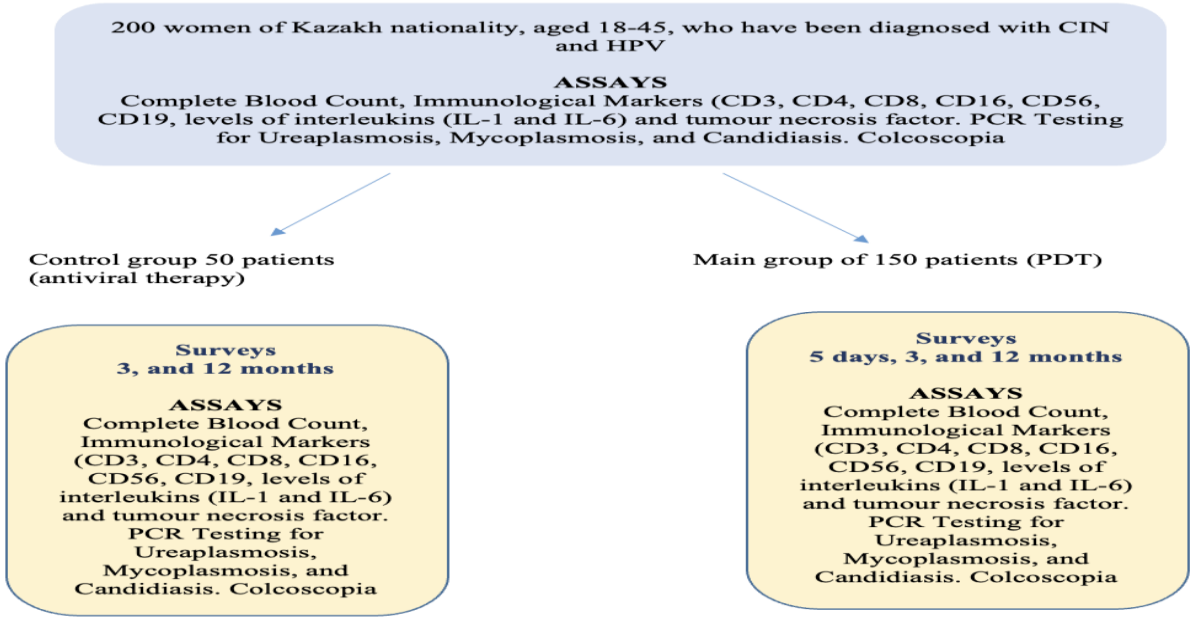


Figure 2 – Algorithm of comprehensive measures for this project

Inclusion criteria for the study:

- Female gender;
- Age 18-48;
- Established diagnosis of CIN;
- Positive PCR test for HPV;
- Kazakh nationality.

Exclusion criteria from the study:

- Age under 18 or over 48;
- Mentally or legally incapacitated individuals, preventing informed consent;
- Pregnant or breastfeeding women;
- Acute gonococcal and non-gonococcal infections of the urethra and lower reproductive tract;
- Severe and decompensated liver and kidney diseases, cardiovascular conditions;
- Autoimmune diseases;
- Cancer;

- Porphyria and other photoallergic conditions;
- Use of anticoagulants.

2.3 HPV Isolation Procedure

The study involved collecting cervical and cervical canal samples using liquid cytology with special staining according to the Papanicolaou method. After a gynaecologist collected the smear using a Rovers Cervex-Brush® with a detachable tip from two points (the epithelium of the endocervix and the exocervix), the tip containing the biological material was placed in a container with a special preservative, BD SurePath. Following the "washing" of the cells through centrifugation, the partially homogenized material was applied in a single layer onto a glass slide for analysis and stained using the Papanicolaou method.

After assessing the amount of material according to the Bethesda classification system (The 2001 Bethesda System terminology), the cytologist provided a conclusion based on the quality of the material. Participants were included in the study if diagnosed with a low-grade squamous intraepithelial lesion (LSIL) (mild dysplasia (CIN 1)) or high-grade squamous intraepithelial lesion (HSIL) (moderate (CIN 2) and severe dysplasia (CIN 3)).

The same material was used for the detection and quantitative assessment of HPV DNA. DNA extraction was performed manually using Extraction-100 kits (Vector Best, Novosibirsk). The analyses were conducted using real-time polymerase chain reaction (PCR) on an open-type CFX 96 amplifier (BioRad). Amplification was carried out using reagent kits for the differential detection and quantitative assessment of HPV DNA for types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, which are associated with high carcinogenic risk.

All necessary operations for analysis and result documentation were performed using the RealBestDiagnostic software program.

2.4 Conducting Whole Exome Sequencing

Participants in the study underwent targeted exome sequencing of DNA. Blood was collected from the cubital vein in a volume of 2 ml using a blood collection system in vacuum tubes with the anticoagulant EDTA and then centrifuged to obtain the cellular component. Genomic DNA was extracted from the cellular residue using the commercial PureLink® Genomic DNA Kits, following the manufacturer's instructions. Candidate genes that may play a potential role in immune protection and inflammation were selected for targeted sequencing based on published studies in the Scopus database. The list of candidate genes also included those with a high degree of damage among biopsy samples from tumors of various localizations.

Sequencing of the amplified fragments was performed using the Ion PGM™ system. A molecular genetic method (NGS technology) was used for semiconductor sequencing of DNA sequences—nucleotide incorporation with proton (H+) detection on a microchip, with read lengths

of 200/400 nucleotides, according to the protocol. Bioinformatics analysis was conducted using the Ion Reporter™ Software, specifically Ion Reporter 5.16, with the "AmpliSeq Exome single sample (Germline)" analysis module. Further bioinformatics processing of the data was performed using filters that included the display of 300 candidate genes, taking into account their relevance to parameters related to the technical aspects of the experiment. Variants with an allele frequency of less than 0.3 and coverage of less than 30 were thus excluded.

2.5 PDT Protocol

PDT is performed from day 5 till day 12 of menstrual cycle. The first stage is intravenous administration of PS-FOTOLON (RUME "Belmedpreparaty", Republic of Belarus), concentration - 1.2 mg/kg body weight for 30 minutes. The drug is certified in the Republic of Kazakhstan (Marketing Authorisation P N015948/01).

The first step involves the intravenous administration of the photosensitizer. Photolon is available as a lyophilized powder for infusion preparation. The vial may contain 25, 50, or 100 mg of the dry active substance—Trisodium Chlorin E6. According to the drug's instructions, it is administered intravenously in doses ranging from 1.0 to 3.0 mg/kg of body weight, depending on the disease type, location, and extent of the pathological process. The dose used in this study was 1.2 mg/kg.

The second stage is fluorescent diagnostics (FD) to monitor photosensitizer accumulation. To do this a LED light source is used (Polironic LLC, Russia), range - 405 nm and a yellow optical filter is installed on a video colposcope (SLV-101 HDm, Lithuania) to cut off ultraviolet radiation and record the red fluorescence of pathologically altered tissues. photosensitizer fluorescence was used to judge the size and extent of the pathological lesion

The third stage is cervical canal irradiation utilizing a diffuser with a working part length of 4 cm and exocervix photoactivation using a macro-lens that transmits radiation from Lahta-Milon laser device (Kvalitek LLC, Russia), wavelength - 662 nm. The following parameters were calculated before photoactivation: power radiant density; and calculation of exposure duration for the diffuser and macro-lens according to previously described formulas [1]. The dose of laser energy to the cervical canal and cervix in patients with LSIL is 250 J/cm^2; in patients with HSIL - 350 J/cm^2. The power density did not exceed 400 mW/cm2 to avoid tissue overheating. Fluorescence examinations are carried out after photoactivation to detect photosensitizer photobleaching in pathological tissues.

2.6 Clinical evaluation

All patients underwent cervical biopsy and liquid-based cytology before treatment. Based on these results, patients were divided into 2 groups according to the Bethesda system: low-grade

squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). Liquid-based cytology was performed 3 months after PDT.

Cumulative response rate (CRR)) was calculated using this formula:

$$\frac{\textit{Number of responders}}{\textit{Total number of participants}} \quad \textit{x 100\%}$$

2.7 Assessment of the Immune Status of Study Participants

To conduct a primary study of immune status and identify significant disruptions in the immune system, the following indicators are analyzed: CD3+ T lymphocytes, CD3+ B lymphocytes, CD3+ CD4+ T helpers, CD3+ CD8+ cytotoxic T lymphocytes, the CD4/CD8 ratio, CD16+ CD56+ natural killers, interleukin 1, interleukin 6, and tumor necrosis factor (TNF-α).

The immunological status of patients was analyzed using flow cytometry, a technique designed to characterize cells in a fluid stream. Parameters are automatically recorded and subjected to computer processing, with the results visualized as graphs. This method allows the investigation of parameters based on light scattering, including forward (cell size) and side scatter (granularity). Further analysis was based on the detection of fluorescent dyes associated with the cells. Monoclonal antibodies or other reagents marked with dyes are used to identify marker molecules on the cell surface (and, with additional cell processing, inside the cell).

When whole blood is added to the reagents, the dye-labeled antibodies in the reagent specifically bind to antigens on the surface of leukocytes. During the measurement (data collection) process, cells pass through a laser beam and scatter its light. Stained cells fluoresce. The data from the scattering and fluorescence signals detected by the instrument provide information about cell size, internal complexity, and the relative intensity of fluorescence.

Conducting the study:

– Vortex the tube with the sample, then place the tube in the analyser’s carousel starting from the first position and securely close the door.

– On the analyser’s screen, enter the patient's ID number in the "Name" field under "Worklist," choose "6-color TBNK" in the "Panel" field, and enter the absolute count of lymphocytes obtained from the patient's complete blood analysis into "Column #3."

– On the top panel of tools, click on the icon with two tubes and an arrow. In the pop-up information window, select "Yes," enter the date, and click "Save."

The research was performed automatically, and the results appeared on the screen; later the results could be opened “Worklist” → “Status”.

Determined indicators of cellular immunity:

– CD3+ T-lymphocytes;

– CD3+CD4+T helper cells;
– CD3+CD8+ cytotoxic T-lymphocytes;
– CD19+ B-lymphocytes;
– CD16+CD56+ natural killer cells;
– CD4/CD8 – immunoregulatory index (CD4/CD8 ratio).

Thus, the immunological method (determining indicators of cellular immunity) of examining patients has been optimized through computerization and visualization of the obtained data in the form of graphs.

2.8 Statistical analysis

Quantitative data were presented as mean values and standard deviations (M±SD) and treated as continuous variables. Qualitative data were represented as frequencies and proportions. Variables were dichotomised: the presence of marriage, miscarriages, and abortions (yes/no). A significance level of $p<0.05$ was considered to determine statistically significant differences. Data analysis was performed using SPSS 26.0 statistical software.

The frequency of polymorphic gene variants between groups was compared using the Pearson χ^2 test. Groups were dichotomized based on HPV carriage. Comparisons were made between HPV 16 carriers and carriers of other HPV types, between HPV 18 carriers and carriers of other types, and between HPV 51 carriers and carriers of other HPV types. These specific types were chosen for analysis due to their widespread prevalence in the population. Statistical calculations were conducted using SPSS 26.0 software.

2.9 Conducting a Systematic Review to Evaluate the Safety and Efficacy of Photodynamic Therapy for Treating Cervical Dysplasia

The systematic review utilized the following inclusion criteria: types of study designs, such as case-control studies, cohort studies, and randomized controlled trials; cases involving cervical dysplasia, squamous intraepithelial lesions, or cervical intraepithelial neoplasia confirmed by biopsy and/or cytology; application of PDT as a mono-therapy or in conjunction with another therapy; all wavelengths and light sources; studies with or without comparators; all clinical outcomes, including but not limited to the HPV eradication rate (HER), the complete response rate (CRR) of lesions, and any adverse effects; and a minimum follow-up period of more than three months. Additionally, we consider dimensions such as country, age, and CIN grade.

After selecting papers three researchers independently reviewed all of them by titles and abstract and obtained relevant papers. In the event of any discrepancies between the reviewers, they were resolved through discussion and consensus to reach a final decision. The relevant data was entered into a spreadsheet for comparative analysis, and if all reviewers agreed, the study was

included in the Mendeley data pool and some duplicated articles were extracted through that tool. During the full-text screening, we excluded studies that did not meet the criteria we had previously specified for inclusion.

The information from the included articles was gathered by three researchers independently using a previously designed data collection form. The following data were extracted from each study: the first author, country, study design, number of patients, median age, follow-up period, type of CIN, intervention, measurement tools, and results with CRR. In the event of any discrepancies between the reviewers, they were resolved through discussion and consensus to reach a final decision.

Joanna Briggs Institute (JBI) tool was applied by three researchers independently to estimate the risk of bias in each separate study. Domains like sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other threats to validity were considered to determine the risk of possible biases. The scale for evaluation consists of three levels of bias: high risk of bias indicates probable bias that seriously weakens confidence in the results if one or more key domains were assessed to be negative. For a low risk of bias which is unlikely to alter the results, all key domains should be positive. If domains raise some doubt about the results and the risk of bias, the last one will be assessed as unclear.

2.10 Ethics

This study adheres to the principles of the Helsinki Declaration of 1964 and Good Clinical Practice. The research was approved by The Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan Local Bioethics with Protocol №3 issued on 9 August 2022 and was conducted under its recommendations. All participants were informed about the study and provided written consent to participate, as well as to use their data for research and educational purposes, ensuring the confidentiality of their personal information.

3 Results of Own Research

3.1 Epidemiological Trends in Screening for Early Detection of Cervical Cancer: A Case Study of a Municipal Hospital

The retrospective analysis of PCR and liquid cytology results performed in the hospital laboratory between 2016 and 2022 revealed an increase in cervical cancer incidence from 314.4 to 341.92 per 100,000, primarily due to a rise in cases among women under 35 years of age. According to our study, in 2016, there were 210 cases of LSIL (2.2%), with 123 being newly identified. In contrast, in 2022, 499 patients were observed (3.48%), with 189 being newly diagnosed.

For HSIL, in 2016, there were 122 patients (1.32% of the population), with 21 newly identified cases. By 2022, this number had increased to 219 (1.53%), with 52 newly diagnosed cases.

Age distribution of newly identified cervical lesions per 100,000 population showed an increase from 0 to 431.03 among patients under 30 years, and from 219.42 to 264.55 for those aged 30-34. There was a significant shift in the predominant age for newly diagnosed lesions from 60 years and older to 40-44 years (from 839.33 to 43.45 and from 196.08 to 491.95, respectively).

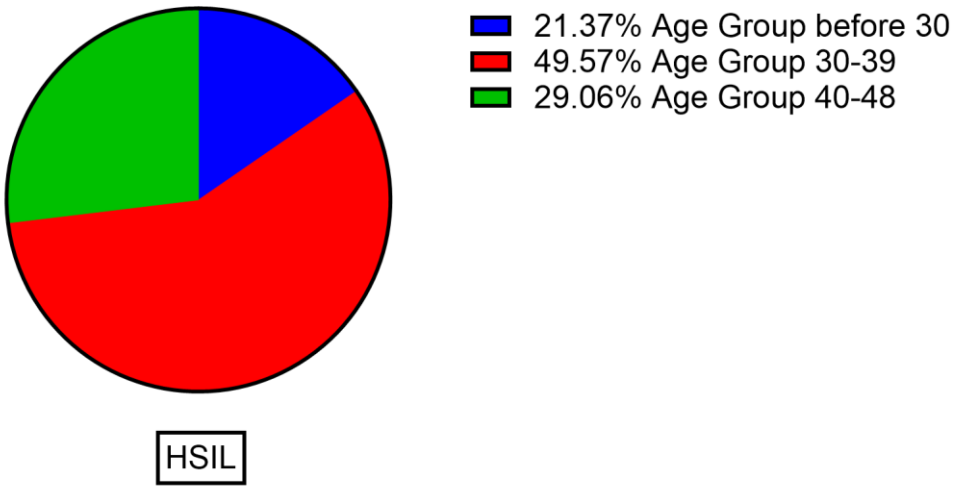
Despite the increase in the coverage of cytological examinations in the hospital from 54% in 2016 to 87% in 2022, there was no corresponding rise in HSIL registrations. Notably, among primary cases of severe dysplasia, the detection of HPV increased from 66.67% to 85.71%, indicating high effectiveness of primary HPV genotype testing.

Additionally, there are epidemiological risks associated with the potential expansion of the influence of other HPV genotypes and an increase in co-infection cases with multiple HPV genotypes in the development of cervical cancer. Specifically, the decrease in the proportion of identified genotypes 16 and 18 in cases of dysplasia and cervical cancer from 97.5% to 79.5% in favor of other genotypes may indirectly indicate a change in the virulence of HPV.

3.2 Clinical and demographic indicators of study participants

According to the study results, the age of patients in the main group ranged from 18 to 48 years, with a median of 36.8±6.4 years (18–48) in the LSIL group and 35.9±6.4 years (27–48) in the HSIL group. Twenty patients were younger than 30 years, 83 patients were aged 30 to 39, and 47 patients were older than 39. LSIL was detected in 123 patients during previous examinations before PDT, while HSIL was found in 27 patients. The distribution of groups is illustrated in Figure 3.

A.



B.

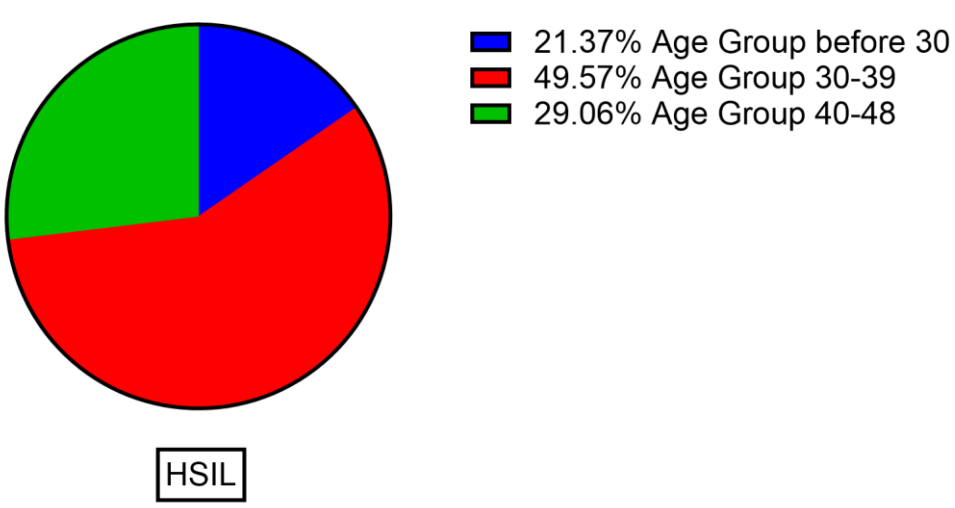


Figure 3 – Grouping of patients by age depending on the severity of dysplasia:
А. Patients with LSIL before PDT; Б. Patients with LSIL before PDT

The average age of patients with LSIL was higher compared to those with HSIL. However, these differences were not statistically significant (p=0.56). Both LSIL and HSIL groups were predominantly composed of married women. In the LSIL group, the ratio of married to unmarried women did not significantly differ, while in the HSIL group, the number of married women significantly exceeded that of unmarried women (p=0.02). The incidence of miscarriages among women with HSIL was much higher than in patients with LSIL; however, these differences were not statistically significant (p=0.28). Medical abortions were performed nearly twice as often among women with HSIL compared to those with LSIL (p=0.05) (Table 1). Thus, married women and those with a history of abortions were more prone to developing HSIL and LSIL, indicating they may be at higher risk for developing cervical dysplasia.

Table 1 – Descriptive statistics based on the severity of dysplasia

	LSIL (n=109)	HSIL (n=22)	p

Continuation of table 1

Mean age (M±SD)	36,8±6,4	35,9±6,4	0,56
In marriage			0,02
Yes	68 (55,1%)	22(81,8%)	
No	55 (44,9%)	5(18,2%)	
Miscarriages			0,28
Yes	28 (22.76%)	12 (44.45%)	
No	95 (77.24%)	15 (55.56%)	
Abortions			0,05
0	101(82,6%)	17(63,6%)	
1	22(17,4%)	10(36,4%)	

The most common type of HPV in both LSIL and HSIL is HPV 16. However, its frequency was higher in cases of severe dysplasia (HSIL). Nonetheless, these differences were not statistically significant (p=0.60). HPV 31 was somewhat less common but was also more prevalent among patients with HSIL (p=0.23). The frequency of HPV types 52 and 58 significantly differed based on the severity of dysplasia, occurring more frequently in severe cases (p=0.01 and p=0.03, respectively). The prevalence of HPV 33, HPV 51, and HPV 56 was also higher among women with HSIL; however, there were no statistically significant differences in the frequency of these HPV types between LSIL and HSIL (p=0.43, p=0.10, p=0.43, respectively). HPV 35 was also more frequently observed in cases of severe dysplasia. In contrast, HPV types 18, 39, and 59 were more commonly found among women with LSIL than those with HSIL, although these differences were not statistically significant (p=0.78, p=0.86, p=0.55). Notably, HPV types 45, 66, and 68 were found exclusively in women with LSIL. The distribution of groups is illustrated in Figure 1.

The analysis focused on the prevalence of leading HPV types and the course of HPV infection in different groups. Among 117 patients with LSIL, 85 (72.6%) had a single HPV type, classified as a mono-infection, with HPV type 16 being the most common. The remaining 32 patients (27.4%) had LSIL and HSIL associated with two or more HPV types, classified as a multi-infection, where HPV type 16 also predominated. Of the 26 patients with HSIL, 12 (46.2%) had a mono-infection, while the others suffered from multi-infections. Overall, among 143 patients, the most frequently detected HPV genotype was type 16 (44%), followed by types 31 (13.2%), 52 (10.5%), and 58 (10.5%). HPV type 18 was found in 6.2% of cases.

The most statistically significant results were observed for HPV types 58 and 52 (p=0.03 and p=0.01, respectively). Consequently, patients with HPV types 58 and 52 fall into the risk group (Table 2).

Table 2 – Descriptive statistics of the distribution of HPV types

Types of HPV	LSIL (n=123)	HSIL (n=27)	p
HPV 16	43(39,5%)	9(40,9%)	0.60
HPV 18	8(7,3%)	1(4,5%)	0.78
HPV 31	14(12,8%)	5(22,7%)	0.23
HPV 33	9(8,3%)	3(13,6%)	0.43
HPV 35	8(7,3%)	2(9,1%)	0.78
HPV 39	6(6,4%)	1(4,5%)	0.86
HPV 45	10(0,9%)	0	
HPV 51	11(1,1%)	1(4,5%)	0,10
HPV 59	9(8,3%)	1(4,5%)	0,55
HPV 56	9(8,3%)	3(13,6%)	0,43
HPV 58	10(0,9%)	6(27,3%)	0,03
HPV 52	11(1,1%)	6(27,3%)	0,01
HPV 66	3(2,8%)	0	
HPV 68	2(1,8%)	0	

In the LSIL study group, 20 patients (17.1%) had a history of treatment with destructive methods for HSIL—4 patients for HSIL and 16 for LSIL. In 17 cases, diathermoelectroconization of the cervix was performed, and 3 patients underwent LEEP excision. All patients showed LSIL in cytological examination results after surgical treatment. The remaining 97 patients in the group had a first-time diagnosis of LSIL and had not received any treatment before FDT.

In the HSIL study group, 6 patients (23.1%) had undergone diathermoelectroconization of the cervix for LSIL between 2009 and 2015, with disease recurrence identified in 2023. HSIL was first diagnosed in 20 patients based on cervical biopsy results.

To investigate potential factors affecting treatment outcomes, we divided patients into groups with primary cervical dysplasia—117 (97 patients with LSIL and 20 with HSIL) and those after surgical treatments—26 (20 patients with LSIL and 6 patients with HSIL). Three months later, the pathological regression rate in the primary dysplasia group was 90.6% (106/117), while the regression rate in the surgical treatment group was 88.5% (23/26). The χ^2 value was 0.11, with $p=0.72 > 0.05$, indicating no statistically significant differences.

3.3 Patient treatment history

In the LSIL group, 20 patients (17.1%) had a history of treatment using destructive methods: 4 cases for HSIL and 16 cases for LSIL. Conization of the cervix was performed in 17 instances, and LEEP excision was done for three patients. All patients showed LSIL in cytological

examinations after surgical treatment. Among the remaining 97 patients in the group, the diagnosis of LSIL was made for the first time, and they had not received any treatment before PDT.

As regards the HSIL group, 6 patients (23.1%) had undergone conization of the cervix for LSIL between 2009 and 2015, with a recurrence of the disease detected in 2023. HSIL was identified based on cervical biopsy results for the first time in 20 patients.

To study possible factors influencing treatment outcomes, we divided patients into groups with primary cervical dysplasia—117 (97 patients with LSIL and 20 with HSIL)—and those who had undergone surgical treatment—26 (20 patients with LSIL and 6 with HSIL). After 3 months, the rate of pathological regression in the primary dysplasia group was 90.6% (106/117), while the regression rate in the group with a history of surgical treatment was 88.5% (23/26). The value of χ^2 was 0.11 (p=0.72), indicating no statistical differences.

Sexually transmitted infections (STIs) were detected in 52 out of 143 patients during preparation for the PDT procedure. Ureaplasma was detected in 37 cases, *Gardnerella vaginalis* in 34 cases, Mycoplasma in 7 cases, and *Chlamydia trachomatis* in 1 case. Patients with STIs were divided into two groups: the first group consisted of 27 individuals with a single type of infection, and the second group included 25 individuals with a combination of different infections. Conservative therapy for these infections was not planned before the start of PDT in this study. The presence of one or more infections was considered only as a factor that might influence the outcomes of cervical dysplasia treatment with PDT.

In the group with only one type of STI, regression of dysplasia occurred in 25 out of 27 patients. The two patients who did not respond to PDT were diagnosed with LSIL. In the group with more than two STIs, disease regression was seen in 19 out of 25 patients, meaning 6 patients did not respond to PDT, 5 of whom had LSIL. Statistical analysis revealed differences in treatment outcomes between these groups, suggesting that having more than two identified STIs before PDT may be a factor reducing treatment effectiveness, with a χ^2 value of 8.92 (p=0.003).

3.4 Development of a method for treating background and precancerous diseases of the cervix with PDT

The essence of the developed technical solution is a method for treating background and precancerous diseases of the cervix, which involves the intravenous administration of a photosensitizer, followed by photomodification of the blood and fluorescent diagnostics, culminating in photodynamic therapy of the cervix and cervical canal. This method is distinguished by the intravenous administration of the photosensitizer "Photolon" at a dose of 1.2 mg/kg of the patient's body weight; 30 minutes later, photomodification of the blood is performed using the "Lachta-Milon" device with a wavelength of 662 nm, an output power of 100 mW, and an exposure time of 30 minutes using a main optical fiber "Polironik" and interchangeable sterile

nozzles for intravenous irradiation. Three hours after the administration of the photosensitizer, under the control of fluorescent diagnostics, subsequent irradiation of the cervical canal is performed using flexible diffusing optical fibers with a scattering length of 4 cm, a power of 500-700 mW, for 14-21 minutes, and the outer portion of the cervix is irradiated using external optical fibers with a power of 600-1200 mW for 6-20 minutes, delivering energy density to the focal point of 250-400 J/cm². After irradiation, fluorescent diagnostics are conducted using an LED illuminator with a wavelength of 400±15 nm to assess the effectiveness of the treatment provided.

The first stage involves the intravenous administration of the photosensitizer. Photolon is available as a lyophilized powder for infusion preparation. The vial may contain 25, 50, or 100 mg of the dry active substance—chlorin E6. According to the drug instructions, it is administered intravenously in doses ranging from 1.0 to 3.0 mg/kg of body weight, depending on the type of disease, its localization, and the extent of the pathological process. The dose used in our study was 1.2 mg/kg.

To prepare the Photolon solution, 50 ml of a 0.9% NaCl solution is added to the powder in the vial and thoroughly mixed until fully dissolved. The following formula is used to calculate the necessary amount of the drug to achieve the desired dose:

$$(N \times m \times V) / A$$

N — the required concentration of the photosensitizer, which in this study is 1.2 mg/kg;

m — the mass of the patient in kg;

V — the volume of the 0.9% NaCl solution used to dissolve the powder in the vial, with a maximum volume of 50 ml;

A — the amount of active substance in the vial, which in this study is 100 mg.

For example, for a patient weighing 70 kg, 42 ml of the drug is needed to achieve a dose of 1.2 mg/kg. The calculated amount of the drug is then dissolved in 200 ml of saline and administered intravenously over 30 minutes.

Three hours after administration, the photosensitizer redistributes in pathological and healthy cells/tissues with maximum contrast. The second stage involves fluorescent diagnostics (FD) to monitor the accumulation of the photosensitizer. A LED light source with a wavelength range of 400±15 nm is used, and a yellow optical filter is placed on the video colposcope to block ultraviolet radiation and record the red fluorescence of pathologically altered tissues. The fluorescence of the photosensitizer is used to determine the size and extent of the pathological lesion.

The third stage involves irradiating the cervix using a diffuser with a working length of 4 cm and photoactivation of the exocervix using a macro lens that transmits radiation from a laser device with a wavelength of 662 nm. Before photoactivation, the following parameters are

calculated: light flux power and exposure time for the diffuser and macro lens according to previously described formulas (13). The laser energy dose for the cervix in patients with LSIL is 250 J/cm²; for patients with HSIL, it is 350 J/cm². The power density does not exceed 400 mW/cm² to avoid overheating the tissues. Fluorescent studies are conducted after photoactivation to detect photobleaching of the photosensitizer in pathological tissues.

The results three months after PDT were evaluated for treatment effectiveness based on standard criteria (WHO), taking into account the dynamics of tumor size changes and morphological control data:

- Complete Effect (CE) — complete disappearance of all disease manifestations, confirmed three months after treatment by laboratory and instrumental diagnostic methods.
- Partial Effect (PE) — visual improvement observed during examinations and colposcopy, confirmed three months after treatment by laboratory and instrumental diagnostic methods, indicated by a transition from severe dysplasia to ASCUS.
- Stabilization (S) — visual improvement noted during examinations and colposcopy, confirmed three months after treatment by laboratory and instrumental diagnostic methods.
- Progression (P) — no visual changes during examinations and colposcopy, or the appearance of new foci observed colposcopically, confirmed by laboratory and instrumental diagnostic methods.

3.4.1 Conducting Systemic Photomodification of Blood

Systemic photomodification of blood is performed 30 minutes after the intravenous administration of the photosensitizer. This involves intravenous irradiation of blood in the cubital vein of the opposite arm using a fiber optic device with an attachment for ILBI on laser machines with a wavelength of 662 nm. The procedure is carried out under the following parameters: laser source power of 100 mW and an irradiation time of 30 minutes.

3.4.2 Development of Fluorescent Diagnostics Technology

In our study, fluorescent diagnostics were performed to determine the tumor boundaries before photodynamic therapy (PDT). Patients were administered a photosensitizer at a dose of 1.2 mg/kg body weight over a period of 30 minutes. Three hours after the administration, the photosensitizer was evenly distributed in the pathological cells, creating maximum contrast.

To track the accumulation of the photosensitizer, we used LED light sources operating in the range of 400±15 nm, along with a yellow filter mounted on the video colposcope. This filter blocks ultraviolet radiation and allows for the registration of red fluorescence from pathologically altered tissues when exposed to ultraviolet light. Thus, we utilized the fluorescence of the photosensitizer to assess the size and extent of the pathological lesion before local photoactivation.

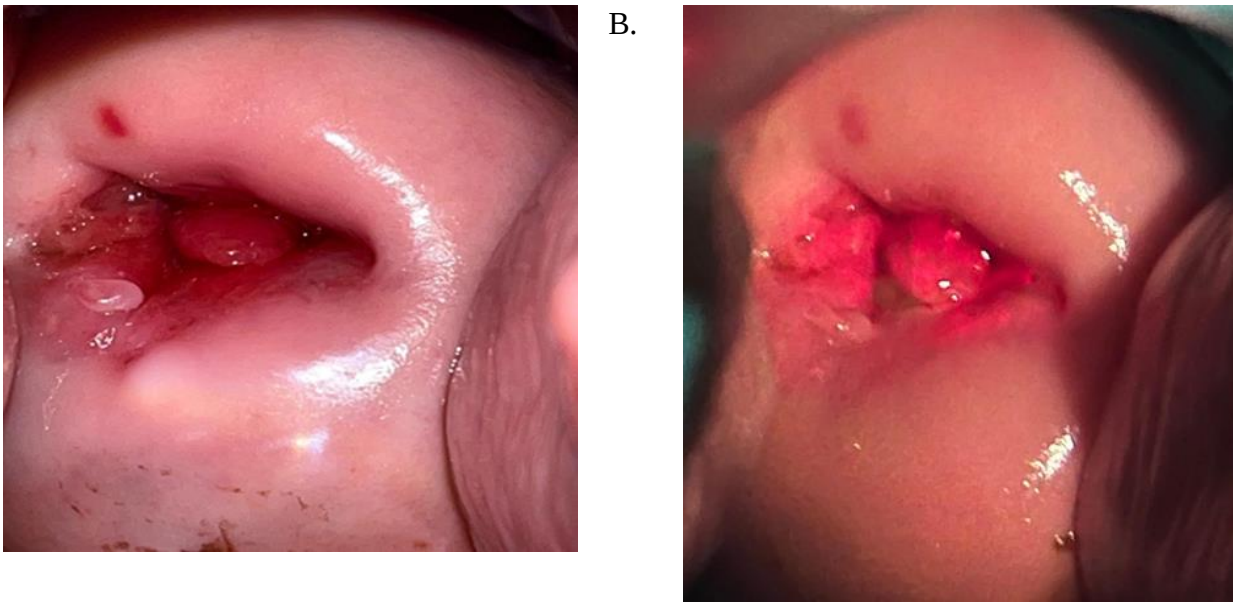
After completing the photoactivation, fluorescent control was conducted to document the effect of complete photosensitizer depletion and evaluate the effectiveness of the radiation dose administered.

3.4.3 Development of a Method for Assessing Fluorescent Diagnostics

In most cases, the fluorescence boundary matched the images from the colposcopy (Figures 4 A, B and 6 A, B). The device used for fluorescent diagnostics in this study (LED 405 nm + yellow optical filter) does not allow for quantitative data on the accumulation of the agent; therefore, the assessment of fluorescence is qualitative, based on the brightness displayed on the video colposcope screen.

In our study, the HSIL group exhibited brighter fluorescence compared to the LSIL group, indicating that the greater the number of atypical cells, the brighter the fluorescence.

A. B.



C. D.

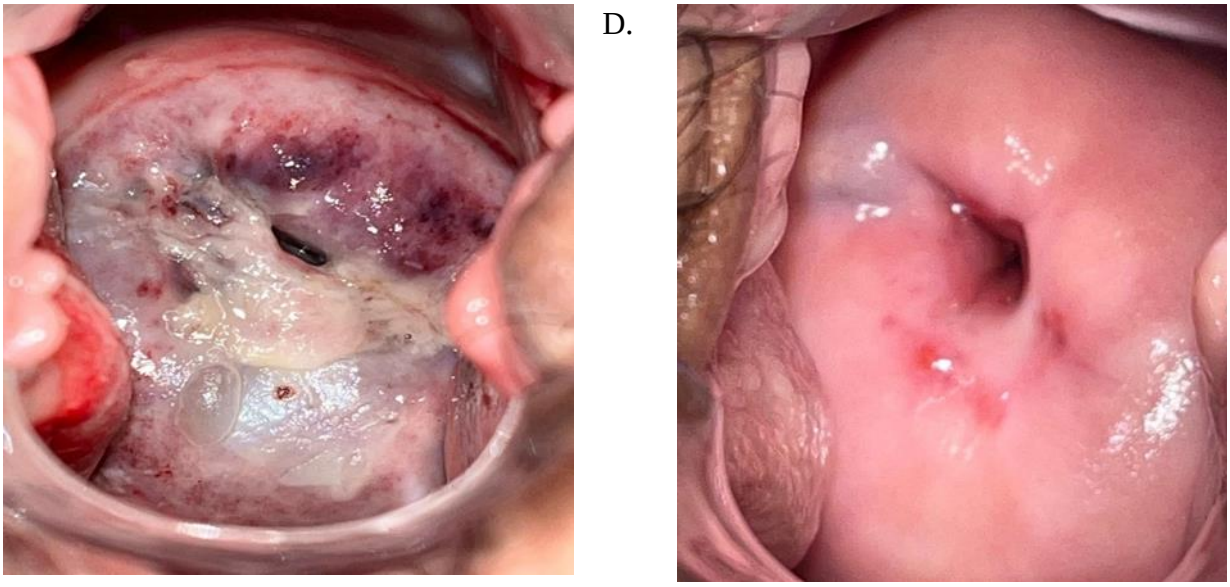


Figure 4 – Colposcopy of a 31-year-old patient

Patient parity – 1. In 2022, the patient underwent conization of the cervix due to LSIL. At the beginning of 2023, during a follow-up examination, a cytological study was conducted, and the diagnosis of LSIL and HPV type 16 was confirmed. A. During the colposcopy of the cervix, the external os is slit-like, and there is a polyp on a stalk with a smooth surface in the lumen of the cervical canal. After treatment with a 3% solution of acetic acid, the abnormal colposcopy picture I: the metaplastic epithelium acquires a delicate acetowhite colour. B. A bright red glow of the cervical canal polyp and around the external os is observed during fluorescence diagnostics. C. On the 5th day after FDT, the cervix is swollen, hyperemic, with a dense fibrinous coating on the surface and petechial hemorrhages. D. At the follow-up examination three months later, complete epithelialization of the cervix with mature epithelium is observed. Cytological study: NILM; according to PCR data from the cervical canal discharge, high-risk oncogenic types of HPV are not detected.

3.4.4 Conducting Local PDT for the Cervix

The patient is positioned on a gynecological chair. Photodynamic therapy (PDT) of the cervix is performed under local anesthesia. Based on the obtained data during fluorescence diagnostics and the prevalence of the pathological process, a protocol for cervical PDT is planned. The following devices are used as sources of laser radiation: "Lachta-Milon" - a laser for PDT. Light guides: a cylindrical diffusion-type light guide with a scattering length of 4 cm and a light guide with micro or macro lenses.

Irradiation of the cervical canal using a flexible cylindrical diffusion-type light guide allows for adequate photoactivation throughout the entire length of the cervical canal. The diameter of the instrument is approximately 2 mm, allowing it to be inserted without the need to dilate the cervical canal. Light exposure is conducted in a pulsed generation mode with a duty cycle of 1.4. The total exposure time ranges from 14 to 21 minutes, depending on the required radiation dose (200-350 J/cm²).

Following this, the vaginal portion of the cervix and, if necessary, the vaginal vaults and walls are subjected to PDT. Irradiation is performed at a distance with a light emission diameter ranging from 1 to 3 cm. The number and size of the treatment fields are selected individually.

To calculate the light dose, the average power is used. The exposure time is determined using the following formula:

$$T = D/P,$$

– T = time of light exposure (minutes);
– DD = required energy density (J/cm²);
– PP = power density (W/cm²).

Power densities during photodynamic therapy (PDT) can range from 100 to 400 mW/cm²; further increases in power density can lead to hyperthermia. The energy density, or radiation dose delivered to the pathological areas, ranges from 150 to 400 J/cm². The output power of the laser source is monitored with an external meter before, during, and after each irradiation session. Control of power density distribution across the irradiated surface is conducted after each adjustment of the laser or light guide and after changing the light guide instrument. To protect the eyes of the physician and surrounding staff during irradiation, special protective goggles designed for use with lasers, supplied by the manufacturer of the laser equipment, are utilized.

3.5 Assessment of the Safety of PDT

The safety of PDT was assessed throughout the entire treatment process. All patients adhered to the light protection guidelines for two days. Complications, such as swelling around the eyes, were reported in two patients, which was linked to a violation of light protection measures. It is recommended to use sunscreen from the first day of photodynamic sensitizer administration and to take antioxidants (vitamins C, E, β-carotene) to prevent phototoxic skin reactions.

Immediately after the procedure, all patients reported dull pain in the lower abdomen and transient burning sensations. Gastrointestinal symptoms (nausea, vomiting) were observed in 27.8% of cases due to intoxication. The early postoperative period was accompanied by an increase in body temperature to 37.8±0.5°C. During PDT, 62.5% (94 patients) reported mild burning, 7.8% (12 patients) experienced moderate burning, 12.7% (19 patients) felt severe burning, while 17% (26 patients) reported no burning sensation. Additionally, 31.7% (48 patients) experienced mild itching, 4.5% (7 patients) reported moderate itching, 5.6% (8 patients) experienced severe itching, while 58.2% (88 patients) had no itching. All these symptoms were temporary and accompanied by spontaneous remission.

Among 150 patients, 2–3 months after PDT, side effects were noted, including endometriosis in 17.2% (26 patients), menstrual cycle disturbances in 21.9% (33 patients), and pulling pains in the lower abdomen in 19.9% (30 patients). However, 41% (62 patients) reported no side effects.

Quality of life assessments for women before and after treatment were evaluated using the QOL-CS questionnaire. Patients were assessed on physical, psychological, social, and spiritual well-being before treatment, 3–7 days after treatment, and at 6 months. Comparison based on these criteria showed that patients' well-being did not suffer.

3.6 Assessment of PDT Effectiveness in Patients with Cervical Dysplasia at 3 and 12 Months

Treatment outcomes were evaluated at 3 and 12 months after PDT. Three months post-treatment, complete regression of the pathological process was observed in 75.6% of patients (93 out of 123) in the LSIL group, while 25 patients (20.3%) still had LSIL. The complete recovery rate for LSIL was therefore 75.6%. In the HSIL group, complete regression was achieved in 92.6% of patients (25 out of 27), with two patients experiencing persistent HSIL. The complete recovery rate in the HSIL group was higher than in the LSIL group at 92.6%. All patients with partial regression underwent repeat PDT.

At the 12-month assessment (as of October 2024), complete recovery was achieved in 101 individuals in the LSIL group (n=105), with 4 patients still having LSIL. The complete recovery rate in this group was 96.1%. In the HSIL group, complete regression was observed in 12 out of 13 patients, with 1 patient remaining with HSIL. The complete recovery rate for the HSIL group was 92.3%. According to the study design, 32 patients (18 LSIL, 14 HSIL) are scheduled to complete relevant tests by the end of 2024.

The complete recovery rate for HPV remission at 12 months was 82.20% (118 out of 97). HPV 16 was detected in the highest number of participants (6), HPV 33 was found in three participants, while HPV 31, 39, 51, and 58 were each found in two participants.

Our study indicates that the likelihood of successful PDT application is higher in HSIL compared to LSIL. This difference may be attributed to lower accumulation of the photosensitizer and a less pronounced photodynamic reaction in patients with LSIL.

3.7 Systematic Review of the Effectiveness and Safety of PDT in Treating Cervical Dysplasia Associated with Human Papillomavirus

The results of our study demonstrate that the complete recovery rate for HPV remission ranges from 66.7% to 92.73%, while for LSIL it fluctuates between 57.1% and 83.3%. The recurrence rate of the disease varied from 3.3% to 8.9% during the observation period of up to 2 years (Table 3).

Table 3 – Research Characteristics

Country	Study design	Intervention	Results, CRR
China [44]	Prospective study	TG - topical PDT with 10 % ALA thermogel (Shanghai Fudan–Zhangjiang Bio- Pharmaceutical Co., Ltd.) with a 635 nm diode laser (LD600-C; Wuhan Yage Photo-	3 month follow up period for HR-HPV remission: CRR=64.10 % in TG vs 24.32 % in CG (x2 = 12.152, $P < 0.01$) • 9 month

Continuation of table 3

		Electronic Co. Ltd, Wuhan, China), light irradiation of 100 J/cm2. Duration: 3 times with 2 weeks interval CG – no treatment	follow-up for HR-HPV remission: CRR=76.92 %% in TG vs 32.40 % in CG (x2 = 15.202, *P* < 0.01) • 9-month follow-up for CIN1 conversion: 83.33 % in TG vs 0 % in CG (X2 = 7.639, *P* < 0.001).
China [45]	Controlled Clinical trial	OG – topical PDT with 20 % 5-ALA; He-Ne laser with 632.8 nm red light, 100 j/cm2; CG – high-frequency electric ion operating treatment. Duration: 4 times with 1 week interval; ALA: 3 h before; PDT: 40–50 min	The 6-month follow-up period for HR-HPV RR: 81.81 % in OG and 52.73 % in CG (x2 = 4.9381, *P* < 0.05); • The 9-month follow-up period for HR-HPV RR: 10.91 % in OG and 7.27 % in CG (x2 = 2.1164, *P* < 0.05); • Total RR of HR-HPV DNA: 92.73 % in OG and 60.0 % in CG (x2 = 4.2615, *P* < 0.05)
Korea [46]	Retrospective study	Photogem intravenously (Moscow, Russia-2 mg/kg) and red laser light with a wavelength of 630 nm (CERALAS; Ceram Optec GmbH, Bonn, Germany), 150 J/cm2. Gr1: only PDT Gr2: PDT + LEEP/Cone Gr3: PDT within 3 months after LEEP/Cone Gr4: PDT after 12 months after LEEP/Cone due to CIN recurrent Duration: Photogem was administered 48 h before laser.	CRR of HR-HPV DNA: • 3-month follow-up period: 89.8 % (44/49); • 12-month follow-up period: 87.0 % (40/46); Total CRR of PDT at 12 months follow up: 98.1 % (52/53)Gr1: CIN2: 100 % (2/2), CIN3: 100 % (6/6), CIS: 80 % (4/5). CRR = 100 % (13/13)
Germany [47]	Prospective, randomized, double-blind, placebo-	TG – HAL vaginal suppositories 100 mg; red coherent light with h wavelength of 633 nm (Biolitec, Germany), 50 J/cm2 CG – placebo	CRR of CIN1 after 6 months: • TG: 57,1 % (20/35 PP) • CG: 25.0 % (4/16 PP) [placebo+PDT:

Continuation of table 3

	controlled, Phase IIa	vaginal suppositories+PDTfollow-up only. Duration: 2 times with 1 month interval; HAL/placebo: 5 h before; PDT: 17 min	40.0 % (4/10) and follow-up group: 0 % (0/6)], $p = 0.040$ CR of HPV: • TG: 73.3 % (11/15 PP) CG: 50 % (5/10 PP) [placebo+PDT: 28.6 %(2/7) and follow-up group: 100 % (3/3)], $p = 0.397$
Germany[48]	Double-blind randomized placebo-controlled, dose-finding study, Phase IIb	topical treatments of HAL hydrochloride 0.2 %, 1 %, 5 % TG1: HAL 5 % TG2: HAL 1 % TG3: HAL 0.2 % CG: placebo Duration: HAL/placebo: 5 h before; automatic illumination for 4.6 h.	There is no statistically significant result in the CIN1 and CIN1/2, also in HAL1 % and HAL0.2 % compared to the placebo group. CRR in CIN2: 3 months: TG - 95 % (18/ 19), Placebo – 57 % (12/ 21), $p = 0.009$ 6 months: TG - 95 % (18/ 19), Placebo – 62 % (13/ 21), $p = 0.021$ CRR in HR-HPV: 3 months: TG - 83 % (5/6), Placebo – 0 % (0/6) 6 months: TG - 83 % (5/6), Placebo – 33 % (2/6) Dose-related response in CIN2+HPV eradication: 6 months: HAL5 % - 84 % (16/19), HAL1 % - 48 %. (14/29), HAL0.2 % - 42 % (8/19), Placebo – 38 % (8/ 21)
Korea [49]	retrospective study	TG: photogem sensitizer and 632 nm diode laser (Biolitec, Ceralas, Germanphotoprintfrin sensitizer and 630 nm diode laser (Diomed, Cambridge, UK), 240 J/cm2. Duration:	CRR in CIN = 95 % Progressive disease: 4.5 % Recurrence: 4.5 % (18 months)

Continuation of table 3

		2 cycles with 1- or 2- months interval (every cycle with 2 days interval)	
Brazil [50]	Randomized Clinical trial	TG: 20 % MAL cream application and CerCa 150 System leds emitting at 630 nm, 80–180 J/cm2 of fluency; CG: only cervix illumination (*n* = 8) or only MAL cream application (*n* = 6) Duration: 2 times with 1-week interval; MAL: 1 or 3 h before PDT.	42 of 56 patients with CIN1 had CRR=75 % for 1 (12.5 %) and 2 (62.5 %) years of follow-up period; CIN1 remained in 5.4 %, CIN2 progression in 8.9 %, CIN1 recurrence in 8.9 % within 2 years after PDT. For CIN 2/3 patients, CRR=90 % after 1 (30 %) and 2 (60 %) years of follow-up period. Placebo group: abstention - 28.57 % and lesion persistence - 14.3 %;
China [51]	Prospective study	TG: 20 % 5-ALA and LED- IB type, wavelength of 633 nm (Wuhan Ya Daylight Electric Technology Co. LTD) and 80 J/cm2. Duration: 3 or 6 times with 1 week interval; PDT: 30 min	CR - 57.14 % for 1 and 2 years of follow-up period CRR in HR-HPV: 3 months = 75.32 % (58/ 77), 6 months = 80.52 % (62/ 77), 12 months = 81.82 % (63/ 77). CRR of CIN1 at 6 months follow up = 88.31 %, at 12 months follow up = 94.81 %
Japan [52]	a clinical trial, Prospective study	20 % 5-Aminolaevulinic acid (5ALA), 633 nm light, 1000–150 J/ cm2 Duration: 2 times with 1–2-week interval	Positive effects = 96.1 % CRR = 70.6 % CR of HPV = 79.4 % Recurrence = 3.7 % (1/51)
China [53]	Retrospective analysis	5ALA and LD600-C photodynamic therapy instrument (Wuhan Yage Optic Electronic nic Technique Co., Ltd.) 635 nm red light wave at 80 mW/cm2. Duration: 3 times with 1–2-	After 6 months of follow-up: the EG = HPV CR - 79.0 %, LSIL reversal rate - 80.6 %, CG = HPV CR - 62.3 %, LSIL reversal rate - 64.2 % (P<0.05)

Continuation of table 3

		week interval; ALA: 3–4 h before; PDT: 30 min	
China [54]	Retrospective analysis	20 % 5-ALA thermosensitive gel (Shanghai Fudan-Zhang Jiang Bio-Pharmaceutical Co, Ltd) and light irradiation at 635 nm and 100 J/ cm2. Duration: 6 times with 1 week interval; ALA: 3 h before; PDT: 30 min	6 months after ALA-PDT: Residual lesion rate – 9.1 % (3/33), p = 0.004 The HPV clearance rate – 66.7 %, p = 0.01 Recurrence rate – 3.3% at 2 years follow up, p = 0.021

Adverse events were reported in 8 (66%) of the studies, with the most common being cervical stenosis, abdominal pain, vaginal pain, and localized edema. A total of five types of local and intravenous applications were primarily used, along with lasers of various wavelengths and intensities. However, all studies demonstrated relatively similar results. According to the data obtained, PDT showed favorable outcomes but did not have a significant impact on the treatment of cervical dysplasia. It is important to emphasize that the effectiveness of PDT for treating cervical dysplasia associated with HPV may vary depending on several factors, including the type of PDT agent used, dosage, duration and frequency of administration, severity and location of lesions, and the body's immunological response.

3.8 post-PDT inflammation and immune cell examination

All patients underwent blood and immune cell examination (absolute number of leukocytes, neutrophils, lymphocytes, monocytes, etc.) and complete blood count (Table 4). As shown in Table 1, patients on the 5th day after PDT exhibited a significant rise in leukocytes, neutrophils, monocytes, and ESR, indicating a notable inflammatory response. In 126 patients, a body temperature exceeding 37 °C was observed on day 2 post-PDT, which resolved on its own within 3-4 days.

Table 4 – CBC parameters in patients before, on day 5 post-PDT and 3 months after PDT

Blood parameter	Reference range	Blood sample period	Patient group			
			LSIL (n=117)		HSIL (n=26)	
Hemoglobin, g/l	120,0-140,0	before PDT	127 (120–137)		129 (127–136)	
		Day 5 post PDT ↓	123 (113–130)	p_1<0,001	122 (117–128)	p_1<0,001
		3 months after PDT	128 (117–134)	p_2=0,001	126 (121–136)	p_2=0,001

Continuation of table 4

Hematocrit, %	32,0-46,0	before PDT	38,4 (35,9–40,5)		38,8 (37,4–41,0)	
		Day 5 post PDT ↓	36,6 (34,4–39,2)	$p_1<0,001$	37,1 (34,8–38,5)	$p_1<0,001$
		3 months after PDT	38,0 (35,9–39,7)	$p_2=0,001$	38,1 (35,6–39,8)	$p_2=0,001$
Erythrocytes, 10^12/l	3,9-4,7	before PDT	4,41 (4,22–4,64)		4,39 (4,28–4,52)	
		Day 5 post PDT ↓	4,27 (3,97–4,5)	$p_1<0,001$	4,12 (4,01–4,36)	$p_1<0,001$
		3 months after PDT	4,38 (4,14–4,65)	$p_2=0,001$	4,16-4,40	$p_2=0,001$
Leukocytes, 10^9/l	4,0-9,0	before PDT	5,94 (5,18–6,94)		5,77 (5,21–6,51)	
		Day 5 post PDT ↑	9,01 (7,69–11,4)	$p_1<0,001$	9,12 (8,16–11,2)	$p_1<0,001$
		3 months after PDT	6,05 (5,18–6,95)	$p_2<0,001$	5,5 (4,86–6,78)	$p_2<0,001$
Neutrophils, 10^9/l	1,7-7,7	before PDT	3,35 (2,87–4,02)		3,21 (2,81–3,51)	
		Day 5 post PDT ↑	6,61 (5,3–8,08)	$p_1<0,001$	6,05 (5,22–7,03)	$p_1<0,001$
		3 months after PDT	3,4 (2,81–4,01)	$p_2<0,001$	3,05 (2,59–4,28)	$p_2<0,001$
Monocytes, 10^9/l	0,0-0,6	before PDT	0,44 (0,39–0,53)		0,43 (0,38–0,47)	
		Day 5 post PDT ↑	0,64 (0,54–0,75)	$p_1<0,001$	0,61 (0,56–0,73)	$p_1<0,001$
		3 months after PDT	0,46 (0,37–0,55)	$p_2<0,001$	0,45 (0,35–0,5)	$p_2<0,001$
Platelets, 10^9/l	150,0-400,0	before PDT	269 (232–319)		270 (235–296)	
		Day 5 post PDT	288 (240–331)	$p_1<0,05$	278 (252–310)	$p_1=0,5$
		3 months after PDT	276 (226–311)	$p_2=0,4$	281 (238–337)	$p_2=0,5$
ESR, mm/h	2,0-20,0	before PDT	15 (8–24)		11 (7–16)	
		Day 5 post PDT ↑	46 (37–70)	$p_1<0,001$	45 (29–55)	$p_1<0,001$
		3 months after PDT	13 (7–21)	$p_2<0,001$	9 (5–20)	$p_2<0,001$

Continuation of table 4

Lymphocytes, %	19,0-40,0	before PDT	31,5 (27,4–35,8)		31,2 (28,9–34,8)	
		Day 5 post PDT ↓	19,3 (16,03–23,4)	p_1<0,001	20,57 (15,8–24,7)	p_1<0,001
		3 months after PDT	31,5 (26,8–36,9)	p_2<0,001	34,7 (26,2–36,8)	p_2<0,001

Note: p1 - differences in blood parameters before PDT and 5 days post PDT.
p2 - differences in blood parameters between results 5 days post PDT and 3 months after the procedure.

Although PDT provides local action, 5 days after inflammation started on the cervix it manifested itself in the form of a systemic reaction which was accompanied by significant neutrocytosis, ESR increase and other indicators of reactive inflammation.

Analysis of cell immunity state was carried out; main cell populations (T-cells, B-cells, natural killers) and T-lymphocyte subpopulations (T-helper cells, CTLs) were determined for the primary study of the immunological status and pro-inflammatory cytokines (IL-1, IL- 6, TNF-α) (Table 5).

Table 5 – Immunological blood indicators in patients before and 5 days post-PDT

Parameter	Reference range	Blood sample Period	Patient group			
			LSIL (n=117)		HSIL (n=26)	
CD3+T-lymphocytes, 10^9/l	0,95-1,8	before PDT	1,32(1,11–1,67)	p_1>0,05	1,51(1,12–1,75)	**p_1<0,05**
		5 days post PDT	1,34(1,1–1,63)		1,46(1,21–1,9)	
CD3+T-lymphocytes, %	55,0-80,0	before PDT	73,8(69,5–78,9)	p_1>0,05	75,43(73–77,8)	p_1>0,05
		5 days post PDT	75(71,2–79)		77,1(74,4–79,7)	
CD3+B-lymphocytes, 10^9/l	0,15-0,4	before PDT	0,19(0,15–0,24)	p_1>0,05	0,18(0,15–0,24)	p_1>0,05
		5 days post PDT	0,18(0,13–0,24)		0,16(0,13–0,22)	
CD3+B-lymphocytes, %	6,0-19,0	before PDT	11(8,54–13,2)	p_1>0,05	9,63(8,74–11,4)	p_1>0,05
		5 days post PDT	10 (8,07–13,0)		9,43(8,32–10)	

Continuation of table 5

CD3+ CD4+T-helper cells, 10^9/l	0,57-1,1	before PDT	0,77(0,63–0,96)	$p_1>0,05$	0,86(0,74–1,04)	$p_1>0,05$
		5 days post PDT	0,78(0,65–1,0)		0,87(0,79–1,09)	
CD3+ CD4+T-helper cells, %	31,0-51,0	before PDT	43,37(38,3–48,4)	$p_1>0,05$	44,9(42,1–50,7)	$p_1>0,05$
		5 days post PDT	44,4(39,5–48,6)		46,5(43,2–49,5)	
CD3+ CD8+ CTLs, 10^9/l	0,45-0,85	before PDT	0,47(0,4–0,64)	$p_1>0,05$	0,5(0,41–0,61)	$p_1>0,05$
		5 days post PDT	0,45(0,36–0,6)		0,49(0,38–0,69)	
CD3+ CD8+ CTLs, %	19,0-35,0	before PDT	27,7(23–32,5)	$p_1>0,05$	27,8(22,5–31,1)	$p_1>0,05$
		5 days post PDT	26,4(23,1–31)		27,4(22,44–30,7)	
CD4/CD8, %	1,5-2,0	before PDT	1,6(1,2–2)	$p_1>0,05$	1,6(1,32–2,2)	$p_1<0,05$
		5 days post PDT	1,7(1,5–2)		1,8(1,5–2,2)	
CD16+ CD56+ NK cells, 10^9/l	0,18-0,42	before PDT	0,21(0,14–0,35)	$p_1>0,05$	0,24(0,21–0,29)	$p_1>0,05$
		5 days post PDT	0,21(0,14–0,31)		0,22(0,17–0,25)	
CD16+ CD56+ NK cells, %	7,0-20,0	before PDT	12(8,28–17,8)	$p_1>0,05$	12,95(9,99–15,2)	$p_1>0,05$
		5 days post PDT	12,6(8,04–16,4)		12,3(9,57–13,8)	
Interleukin-1, pg/ml	0–11,0	before PDT	0,098(0,0–1,33)	$p_1<0,001$	0,098(0,098–1,12)	$p_1<0,001$
		5 days post PDT	0,725(0,098–1,31)		0,714(0,098–0,89)	
Interleukin-6, pg/ml	0,0-3,4	before PDT	1,32(0,46–2,46)	$p_1<0,001$	1,18(0,22–2,08)	$p_1<0,001$ $p_3<0,01$
		5 days post PDT	4,15(0,098–10,41)		8,41(0,6–12,66)	

Continuation of table 5

TNF-α, pg/ml	0,0-6,0	before PDT	0,098(0,0–0,71)	p_1<0,001	0,16(0,098–1,15)	p_1<0,001 p_3<0,01
		5 days post PDT	0,67(0,098–2,09)		3,01(0,098–4,61)	

Note:

p1 - differences in parameters before PDT and 5 days post PDT.

p3 — differences in parameters between LSIL and HSIL groups on day 5 post PDT.

There were no statistically significant differences in the levels of circulating immune complexes in the C3 and C4 complement components of peripheral blood in patients on day 5 post-PDT. However, systemic reactions were associated with increased levels of traditional inflammatory indicators and proinflammatory cytokines. A statistically significant difference between patient groups was observed using the chi-square test. Specifically, IL-6 and TNF-α levels on day 5 post-PDT were higher in the HSIL group compared to the LSIL group. The χ^2 value for IL-6 was 8.89, and for TNF-α, it was 9.27, with a significance level of $p<0.01$.

Work carried out on the example of one patient:

Patient HWOC020.

Clinical diagnosis: severe cervical dysplasia. HPV 45+.

Operation: 05/12/2023 photodynamic therapy. She underwent the manipulation moderately. From the anamnesis: Pregnancies: 2, Childbirth: 1, Abortions: 1. Contraception: barrier. Gynaecological operations: none. Planning a pregnancy - yes.

Cytological examination of smears stained by Papanicolaou. The specimen was prepared using liquid cytology. The quality of the drug is adequate. In the submitted material, against the background of neutrophilic leukocytes, cells of the superficial, intermediate layers of multilayered squamous epithelium and metaplastic cells with signs of low-grade squamous intraepithelial lesions were found. Among them, in clusters, there are glandular epithelial cells without signs of atypia. Conclusion: "Features of Low-Grade Squamous Intraepithelial Lesion (LSIL)."

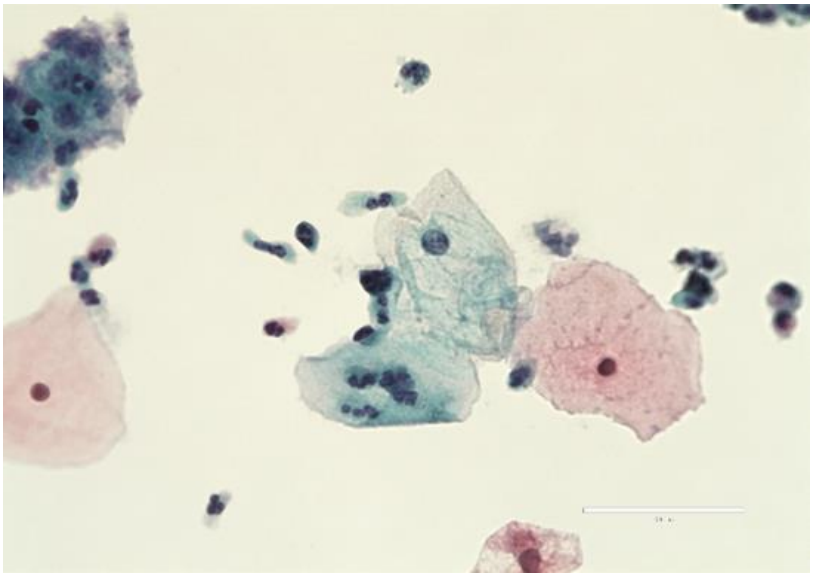
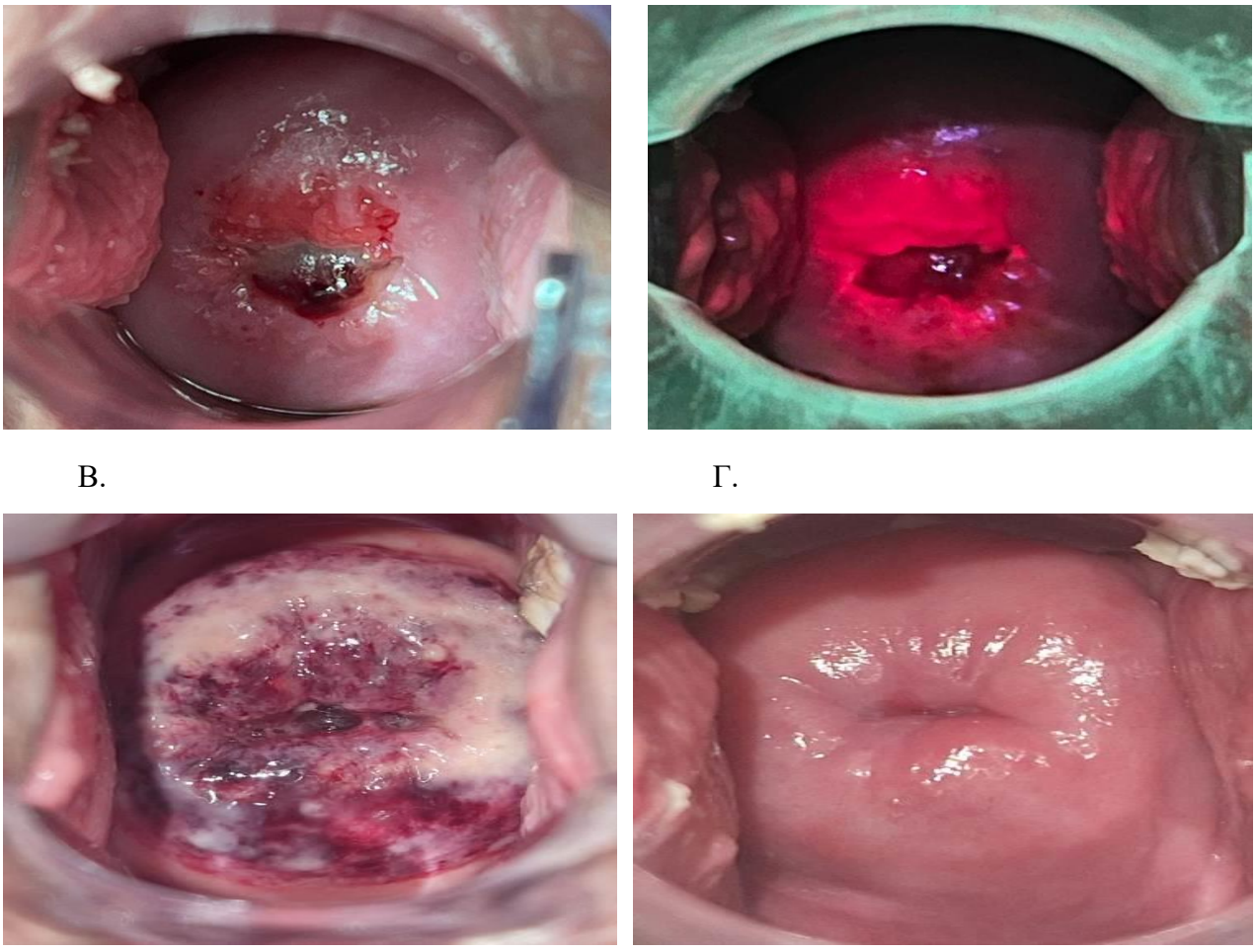


Figure 5 – Cytological examination of smears stained by Papanicolaou. Signs of LSIL

А. Б.



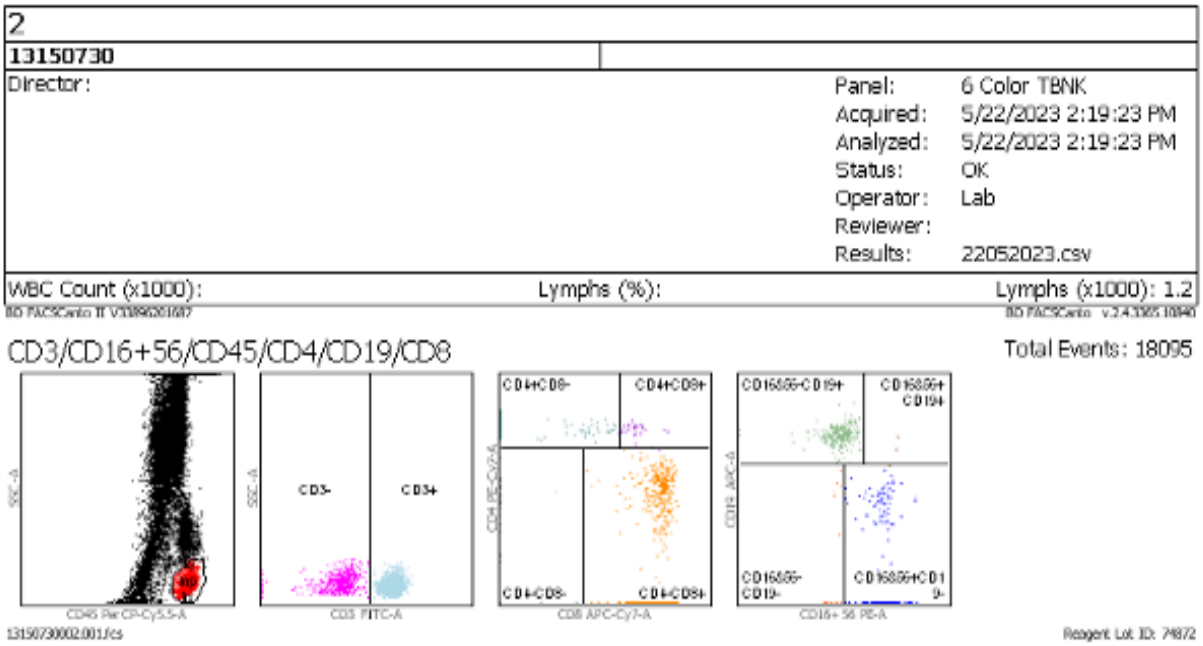
В. Г.

Figure 6 – Colposcopy of a 31-year-old patient.

Medical history: patient parity - 1, did not visit a gynecologist for 4 years. The patient got a pap smear and was tested for HPV (PCR method) at periodic screening in February 2023, which revealed moderate cervical intraepithelial neoplasia - HSIL and highly oncogenic HPV 31 type. A. Standard colposcopy reveals areas of acetowhite epithelium at the 12 o'clock position, with atypical epithelium appearing as a mosaic and punctation (capillary projections appear as fine, uniformly spaced red spots). Pearly clusters are observed at the edges of the transformation zone, which are clear indicators of HPV infection. B. Fluorescent colposcopy visualizes lesions that are bright raspberry in colour (such patterns are often seen in cases of severe dysplasia and cancer), complementing and enhancing traditional colposcopy examination. C. On the 5th day, extensive dense scabbing is observed following photodynamic therapy. D. The cervix after PDT at 3 months.

The vascular response of the cervix, characterized by cyanosis, is observed immediately after PDT (Fig.6). Subsequently, the inflammatory reaction intensifies in the surrounding tissues, manifesting as fibrin formation on the cervix by the 5th-day post-PDT, which leads to necrosis of the affected areas. Approximately 18–21 days after PDT, the necrotic eschar is rejected, and epithelial regeneration begins. Thus, during colposcopic examination using Photolon, no differentiated cells were found over time, indicating the effectiveness of laser technologies with photosensitization.

The immune status of the patient was assessed using a panel of T and B lymphocytes and analyzed with a fluorescent cytometer.



2

13150730

Director:

Panel: 6 Color TBNK
Acquired: 5/22/2023 2:19:23 PM
Analyzed: 5/22/2023 2:19:23 PM
Status: OK
Operator: Lab
Reviewer:
Results: 22052023.csv

WBC Count (x1000): Lymphs (%): Lymphs (x1000): 1.2

CD3/CD16+56/CD45/CD4/CD19/CD8

Total Events: 18095

Parameter	Percent	Value/AbsCnt
Lymph Events		2509
CD3+	80.39	964.69
CD3+CD8+	37.58	451.02
CD3+CD4+	37.90	454.84
CD3+CD4+CD8+	1.08	12.91
CD16+CD56+	7.45	89.44
CD19+	11.48	137.74
CD45+		1200
4/8 Ratio		1.01

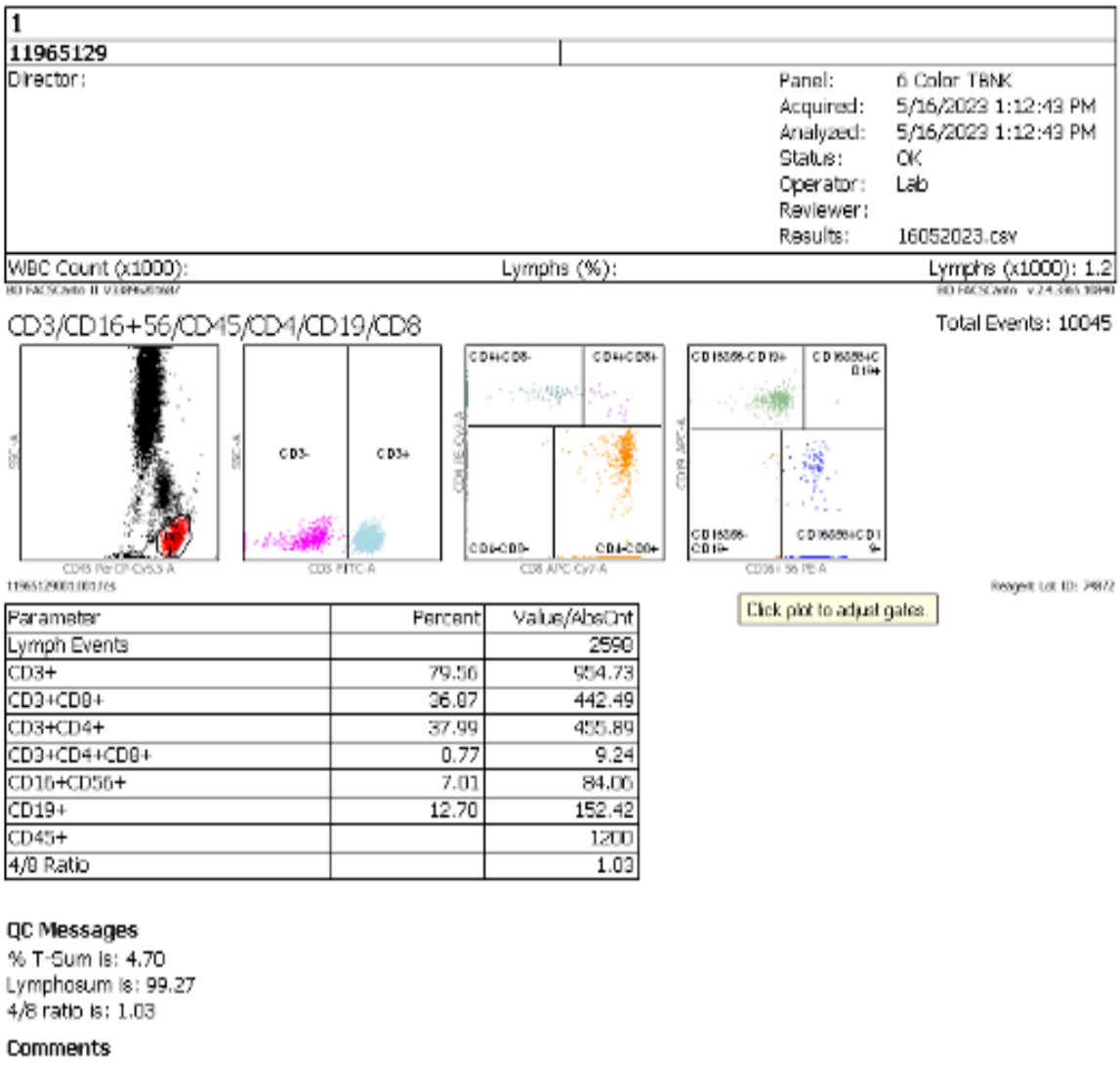
QC Messages

% T-Sum is: 4.90
Lymphosum is: 99.32
4/8 ratio is: 1.01

Comments

Figure 7 – Immunological picture of the patient before PDT

In the examined peripheral blood sample, the lymphocytes are within the normal range in both absolute and relative values, constituting 27.2% (1.30 x 10^9/L). The subpopulation composition of lymphocytes includes all T-V-NK subpopulations. The total number of T-lymphocytes (CD3+) is within the normal range. However, there is an observed imbalance in the content of the major T-cell populations (CD3+CD4+/CD3+CD8+), which is due to fewer T-helper cells (CD3+CD4+). The number of B-lymphocytes (CD3-CD19+) and natural killer cells (CD16+CD56+) is within the normal range.



Parameter	Percent	Value/AbsCnt
Lymph Events		2590
CD3+	79.56	954.73
CD3+CD8+	36.87	442.49
CD3+CD4+	37.99	455.89
CD3+CD4+CD8+	0.77	9.24
CD16+CD56+	7.01	84.06
CD19+	12.70	152.42
CD45+		1200
4/8 Ratio		1.03

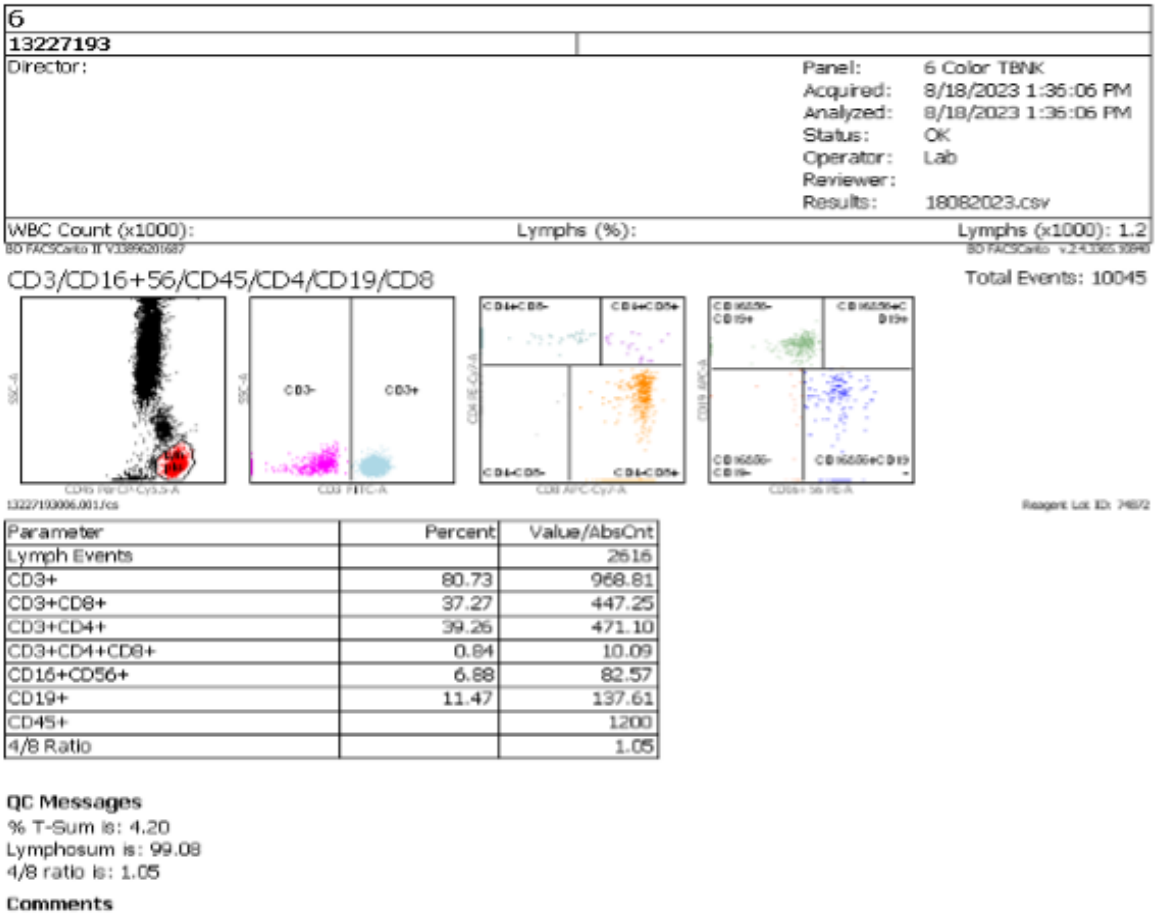
QC Messages

% T-Sum is: 4.70
Lymphosum is: 99.27
4/8 ratio is: 1.03

Comments

Figure 8 – Immunological picture of the patient on the 5th day after PDT

In the examined peripheral blood sample, the absolute count of lymphocytes remains within the normal range at 1.42 x 10^9/L, but the relative percentage has decreased to 15.8%. The total number of T-lymphocytes (CD3+) and T-helper cells (CD3+CD4+) are within the normal range. However, the immunoregulatory index (CD4/CD8) remains reduced at 1.4.



Parameter	Percent	Value/AbsCnt
Lymph Events		2616
CD3+	80.73	968.81
CD3+CD8+	37.27	447.25
CD3+CD4+	39.26	471.10
CD3+CD4+CD8+	0.84	10.09
CD16+CD56+	6.88	82.57
CD19+	11.47	137.61
CD45+		1200
4/8 Ratio		1.05

Figure 9 – Immunological picture of the patient 3 months after PDT

In the peripheral blood sample under study, the number of lymphocytes and their subpopulation composition were within normal limits. The immunoregulatory index (CD4/CD8) remains reduced to 1.3. Conclusion: The immunoregulatory index depends on the CD4/CD8 ratio; a reduced value indicates a deterioration in the functioning of the immune system and indicates that the function of protective cells in the body is weakened. This is usually observed in pathologies accompanied by immunodeficiency: infectious diseases, congenital immunodeficiency, any protracted and chronic diseases.

Thus, during a colposcopic study using Photolon, no differentiated cells were detected over time, which indicates the effectiveness of laser technologies with photosensitization.

3.9 Results of Targeted Exome Sequencing of DNA from Study Participants

3.9.1 Frequency of Genes and Polymorphic Variants of HLA System Genes

According to the results of exome sequencing, four significant genes (HLA-DQB1, HLA-DQA2, TAP2, HLA-C) were identified concerning the persistence of three types of HPV (16, 18, 51) in cervical intraepithelial neoplasia (CIN). The allelic polymorphism of each of the significant genes was investigated (see Table 2).

In our research, HLA-DQB1 gene, the most common polymorphic variants were found among HPV 16 carriers, while variants of sequence variability in the HLA-DQB1 gene were rarely observed in HPV 18 carriers.

The frequency of changes in the HLA-DQA2 gene was higher among HPV 18 carriers. Specifically, polymorphic variants such as rs62619945 (C/A), rs199931222 (G/A), and rs200904145 (A/G) were recorded (Table 6).

Changes in the TAR2 gene were most frequently observed in women with HPV 51. Polymorphic variants rs241447 (T/C), rs241449 (C/A), and rs241448 (A/G) occurred in nearly 9% of HPV 51 carriers, while they were present in only 5% of HPV 16 carriers. Sequence variability in the TAR2 gene was virtually absent in HPV 18 carriers.

Regarding the HLA-C gene, polymorphic variants rs2308585 (G/A, C, T), rs1050326 (C/A, G, T), rs2308618 (G/A, C), and rs241448 (A/G) were found in HPV 16 carriers. Although only rs1050326 (C/A, G, T) and rs241448 (A/G) were observed in HPV 18 carriers, their frequency was higher compared to the same polymorphic variants in HPV 16 carriers. In HPV 51 carriers, polymorphic variants rs1050326 (C/A, G, T), rs2308618 (G/A, C), and rs241448 (A/G) were present, with the frequency of rs1050326 (C/A, G, T) significantly higher compared to its frequency in HPV 16 and 18 carriers.

Table 6 – Frequency of occurrence of genes and polymorphic variants of genes of the HLA system identified by whole exome sequencing

Gene/polymorphism	HPV16 (n=52)	HPV18 (n=9)	HPV51 (n=12)
HLA-DQB1			
rs1063322	7(13,2%)	0	1(8,33%)
rs9273527	5(9,43%)	0	1(8,33%)
rs74188963	1(1,89%)	0	0
rs9273511	0	0	1(8,33%)
rs3213490	2(3,77%)	0	1(8,33%)
HLA-DQA2			
rs62619945	10(18,9%)	5(55,6%)	5 (41,7%)
rs2051600	3 (5,66%)	0	1(8,33%)
rs115121776	7 (13,2%)	3()	3 (25%)
rs9276437	5 (9,43%)	0	3 (25%)
rs138296677	7 (13,2%)	0	1(8,33%)
rs199931222	14 (26,4%)	4(44,4%)	3(25,0%)

Continuation of table 6

rs142901825	8 (15,1%)	0	1(8,33%)
rs1129956	16 (30,2%)	3(33,3%)	4 (33,3%)
rs34730447	1 (1,89%)	0	2 (16,7%)
rs200904145	6 (11,3%)	4(44,4%)	4 (33,3%)
rs201291459	1(1,89%)	0	2 (16,7%)
HLA-C			
rs2308585	2 (3,77%)	0	1 (8,33%)
rs1050326	2 (3,77%)	1(10%)	2 (16,7%)
rs2308618	3 (5,66%)	0	0
rs1131104	4 (7,54%)	1(10%)	1 (8,33%)

3.9.2 Comparison of Sequence Variability Frequencies in HLA Genes Among Patients with Dysplasia Based on the presence of HPV 16

When comparing the frequency of polymorphic variants in the HLA-DQB1 gene through whole-exome sequencing, an overrepresentation of variants rs1063322 and rs3213490 was observed among HPV 16 carriers compared to patients with other HPV variants (Table 7). However, these differences were not statistically significant. Other sequence changes identified in the gene (rs9273527, rs74188963, rs9273511) were more prevalent in patients with different HPV variants. The frequency of polymorphic variants in the HLA-DQA2 gene among HPV 16 patients did not significantly differ from the frequency of polymorphic variants in carriers of other HPV types.

Despite the highest frequency of sequence changes in the HLA-C gene identified through whole-exome sequencing being attributed to HPV 16, these differences were also not statistically significant.

Table 7 – Comparison of the frequency of occurrence of sequence variability variants in the genes of the HLA system in patients with dysplasia depending on the carriage of HPV 16

Gene/polymorphism	HPV 18 carriers (n=53)	Carriers of other HPV variants (n=200)	p
HLA-DQB1			
rs1063322	7	4	0,10
rs9273527	2	3	0,98
rs74188963	1	1	0,78
rs9273511	0	4	0,09

Continuation of table 7

rs3213490	2	1	0,35
HLA-DQA2			
rs62619945	10	15	0,96
rs2051600	3	2	0,36
rs115121776	7	12	0,73
rs9276437	5	7	0,93
rs138296677	7	8	0,60
rs199931222	14	14	0,25
rs142901825	8	9	0,55
rs1129956	16	23	0,93
rs34730447	1	3	0,52
rs200904145	6	11	0,64
rs201291459	1	3	0,52
HLA-C			
rs2308585	2	1	0,35
rs1050326	2	6	0,36
rs2308618	3	2	0,36
rs1131104	4	4	0,57

3.9.3 Comparison of Sequence Variability Frequencies in HLA Genes Among Patients with Dysplasia Based on the presence of HPV 18

In the analysis of the frequency of polymorphic variants in the HLA-DQB1 gene through whole-exome sequencing, it was found that sequence changes such as rs1063322, rs3213490, rs9273527, rs74188963, and rs9273511, which were present in other HPV carriers, were virtually absent among HPV 18 carriers (Table 8).

Regarding the HLA-DQA2 gene, the frequency of the polymorphic variant rs62619945 was significantly higher among HPV 18 carriers (55.6%) compared to carriers of other HPV variants (20.5%), with these differences being statistically significant (p=0.01). A similar statistically significant prevalence was observed for the polymorphic variant rs200904145 among HPV 18 carriers (44.4%, p=0.008). Other polymorphic variants, such as rs2051600, rs9276437, rs138296677, rs142901825, rs34730447, and rs201291459, were rarely found among HPV 18 carriers (Table 4).

In examining the frequency of polymorphic variants in the HLA-C gene, it was noted that sequence changes such as rs1050326 and rs1131104, which were present in other carriers,

appeared only in isolated cases among HPV 18 carriers. Additionally, polymorphic variants rs2308585 and rs2308618 were not found in our sample of HPV 18 carriers.

Table 8 – Comparison of the frequency of occurrence of sequence variability variants in the gene in patients with dysplasia depending on the carriage of HPV 18

Gene/polymorphism	HPV 18 carriers (n=9)	Carriers of other HPV variants (n=200)	p
HLA-DQB1			
rs1063322	0	11	0,32
rs9273527	0	5	0,51
rs74188963	0	2	0,78
rs9273511	0	4	0,56
rs3213490	0	3	0,61
HLA-DQA2			
rs62619945	5 (55,6%)	25 (20,5%)	0,01
rs2051600	0	5	0,51
rs115121776	3	16	0,15
rs9276437	0	12	0,30
rs138296677	0	15	0,24
rs199931222	4	24	0,14
rs142901825	0	17	0,20
rs1129956	3	36	0,99
rs34730447	0	4	0,56
rs200904145	4 (44,4%)	13 (10,7%)	0,008
rs201291459	0	4	0,56
HLA-C			
rs2308585	0	3	0,61
rs1050326	1	7	0,59
rs2308618	0	5	0,51
rs1131104	1	7	0,59

3.9.4 Comparison of Sequence Variability Frequencies in HLA Genes Among Patients with Dysplasia Based on the presence of HPV 51

In the analysis of sequence changes in the HLA-DQB1 gene among HPV 51 carriers, it was found that polymorphic variants such as rs1063322, rs9273527, rs9273511, and rs3213490, which were present in other HPV carriers, occurred only in isolated cases in HPV 51 carriers

(Table 5). The polymorphic variant rs74188963 was virtually absent in our sample of HPV 51 carriers.

When comparing the frequency of polymorphic variants in the HLA-DQA2 gene, it was observed that the frequency of the polymorphic variant rs9276437 was highest among HPV 51 carriers (25%) compared to carriers of other HPV variants (6.6%), with these differences being statistically significant (p=0.03). A similar statistically significant prevalence was noted for polymorphic variants rs200904145, rs34730447, and rs201291459 among HPV 51 carriers (Table 5).

In contrast, the frequency of polymorphic variants in the HLA-C gene among HPV 51 patients did not significantly differ from that of carriers of other HPV variants (Table 9).

Table 9 – Comparison of the frequency of occurrence of sequence variability variants in the gene in patients with dysplasia depending on the carriage of HPV 51

Gene/polymorphism	51 carriers (n=12)	Carriers of other HPV variants (n =200)	p
HLA-DQB1			
rs1063322	1	10	0,99
rs9273527	1	4	0,39
rs74188963	0	2	0,65
rs9273511	1	3	0,27
rs3213490	1	2	0,14
HLA-DQA2			
rs62619945	5 (41,7%)	25 (20.5%)	0,11
rs2051600	4	1	0,39
rs115121776	3	16	0,28
rs9276437	3 (25%)	8 (6,6%)	0,03
rs138296677	1	14	0,72
rs199931222	3	25	0,75
rs142901825	1	16	0,62
rs1129956	4	35	0,78
rs34730447	2 (16,7%)	2 (1,6%)	0,004
rs200904145	4 (30,7%)	13(10,6%)	0,03
rs201291459	2 (16,7%)	2 (1,6%)	0,004
HLA-C			
rs2308585	1	2	0,14

Continuation of table 9

rs1050326	2	6	0,11
rs2308618	0	5	0,47
rs1131104	1	7	0,74

Среди 4 генов (HLA-DQB1; HLA-DQA2; TAP 2; HLA-C), значимых в персистировании инфекции 3 типов (16,18,51) ВПЧ высокого риска у казахских женщин с дисплазией шейки матки, полиморфизмы rs62619945, rs200904145, rs347304447, rs9276437, rs2012911459, rs241447 гена HLA-DQA2 продемонстрировали значимое влияние.

3.10 The Algorithm for Managing Patients With Cervical Dysplasia

Risk management for the development of cervical cancer in HPV infection includes a series of sequential measures supported by objective evidence of their effectiveness for various health levels: preventive measures, PDT, and invasive interventions.

Within the framework of healthcare management, the following stages are outlined: screening, preventive rehabilitation, managed oncogenesis, sub-managed oncogenesis, and unmanaged oncogenesis (Figure 10).

– Screening Stage: The goal is to identify the risk of developing cervical cancer. Starting at age 20, it is recommended to perform PCR testing for 12 high-risk HPV genotypes every four years. If the result is positive, cytological evaluation should follow.

– Preventive Rehabilitation Stage: This stage focuses on patients without LSIL who have tested HPV-positive two or more times in the past 24 months. The aim is to prolong the "pre-dysplasia" period. Key tasks include correcting reversible immune disorders through behavioral and preventive measures, as well as establishing a healthy cervical microbiota to prevent HPV invasion into the epithelium. Changes in the cervical epithelium and monitoring of qualitative and quantitative HPV genotype indicators are conducted every six months.

– Managed Oncogenesis Stage: Attention is directed toward patients with cervical dysplasia and HPV. The primary objective is to limit HPV invasion into the epithelium. Cervical cytology and qualitative HPV assessment are performed quarterly. If high-risk genetic markers are present and there is progression in cytological findings, the possibility of photodynamic therapy (PDT) is considered.

– Sub-Managed Oncogenesis Stage: Patients with HSIL and HPV face the challenge of slowing HPV integration into the host DNA. Qualitative HPV assessment is also conducted quarterly. A comprehensive PDT procedure is performed, with repetition if necessary. If high-risk genetic markers are identified, oncological surveillance is added three months after PDT, and a decision regarding surgical treatment is made.

– Unmanaged Oncogenesis Stage: This stage focuses on patients with HSIL. The goal is to maintain the immune function of the cervix for as long as possible. The main activities during this period include oncological surveillance and timely invasive intervention. PDT plays a supportive role, with the possibility of reapplication if there is improvement in cytological findings or deterioration in the patient's condition.

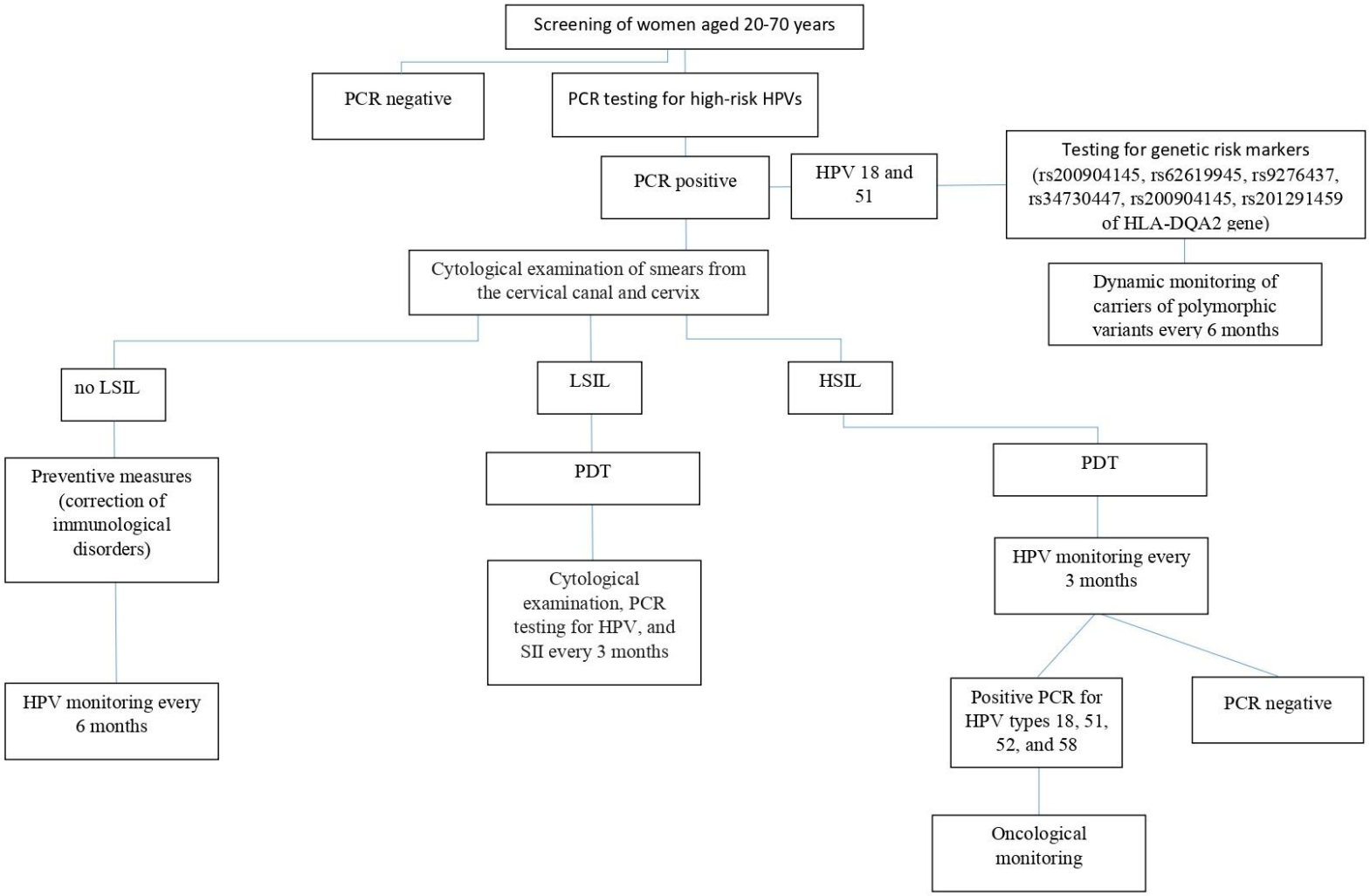


Figure 10 – Algorithm for the management of patients with high-risk HPV types

CONCLUSION

For the first time in Kazakhstan, photodynamic therapy (PDT) was used with a combination of local and systemic laser treatment for HPV-associated precancerous diseases of the cervix. A significant advantage of PDT is the preservation of the anatomical structure of the cervix, as demonstrated in our study. Moreover, PDT is comparable to accepted invasive and surgical treatment methods in terms of long-term remission rates, while the consequences of destructive treatment methods can lead to obstetric complications.

During the implementation of the program, several factors were identified that should be considered when prescribing PDT for women with cervical lesions due to HPV:

1. The example of a well-organized cohort demonstrated additional opportunities for effective cervical cancer screening. It became evident that the rise in cervical dysplasia cases is predominantly due to newly identified cases in women under 35, indicating the need to include women aged 20 and older in screening. Given the reproductive age, expanding access to non-invasive treatment methods is becoming increasingly important. The higher effectiveness of HPV genotype testing compared to cytology suggests its priority as a primary screening test.

2. There is a clear need to adjust the cervical cancer screening strategy in Kazakhstan. Additionally, changes in the trends of cervical dysplasia associated with HPV may generate new scientific hypotheses for further research, the results of which could shift the focus of clinical practice.

3. According to epidemiological observations, married women and those with a history of abortions were most prone to developing HSIL and LSIL and may be included in the risk group for cervical dysplasia. Overall, among 150 patients, the most frequently detected HPV genotype was type 16, followed by types 31, 52, and 58. However, HPV types 52 and 58 were more commonly found in HSIL cases ($p=0.01$ and $p=0.03$, respectively).

4. The evaluation of complete regression of the pathological process 12 months after PDT showed that the rates of complete recovery in the LSIL and HSIL groups were 96.1% and 92.3%, respectively. In contrast, our systematic analysis of the effectiveness of PDT in other countries revealed that the complete recovery rate for the LSIL group ranged from 57.1% to 83.3%. In global practice, the organic acid 5-aminolevulinic acid (5-ALA) and its derivative, hematoporphyrin-levulinic acid (HAL), are used as photosensitizers. The advantages of the photosensitizer we used, Photolon, compared to 5-ALA include a significantly higher quantum efficiency of singlet oxygen (Photolon – 0.7; 5-ALA – approximately 0.2-0.3). The peak absorption band of Photolon is around 662 nm, while for 5-ALA it is 630 nm, which facilitates deeper light penetration. Additionally, the series of chlorin photosensitizers exhibits greater endothelial tropism compared to its predecessor, protoporphyrin IX, making chlorin compounds more effective for treating precancerous conditions

and tumors due to their more pronounced vascular effects. However, it is worth noting that statistical analysis revealed differences in our treatment outcomes between the LSIL and HSIL groups, indicating that the presence of more than two diagnosed STIs prior to PDT may be a factor that reduces treatment efficacy.

5. The study evaluated the impact of the systemic inflammatory response triggered by the photosensitizer after PDT on HPV-associated cervical dysplasia. Systemic reactions observed five days post-PDT were linked to elevated levels of traditional inflammatory markers and pro-inflammatory cytokines, indicating an active immune response against atypical cells. Among the factors influencing immune regulation, lymphocytes play a key role in resolving persistent infections. Therefore, the ratio of platelets, neutrophils, and lymphocytes (systemic immune-inflammation index, SII) may serve as a reliable and cost-effective biomarker for predicting the risk of recurrence after PDT, particularly in cases of high-grade cervical lesions.

6. For the first time in the world, we investigated genetic variations in the HLA system in patients with cervical dysplasia based on the type of HPV. The results of our study may suggest the involvement of HLA-DQA2 gene alleles in the persistence of HPV 18 and HPV 51 and the immune response to the virus in women of Kazakh ethnicity.

The algorithm we developed for managing patients with high-risk HPV types is a scientific product for practical use and has significance for shifting the focus of clinical practice. This algorithm is based on the sequence of biological processes that lead from an unstable immune response to HPV to irreversible pathological changes in cervical cancer. Such an approach allows for the management of the duration of the "pre-disease" period.

Implementing this in medical practice can reduce the burden of HPV in the medium term through early intervention at the level of risk factors. Understanding the causes of abnormal immune responses based on genetic risk factors may broaden strategies for managing HPV infections and associated diseases. Additionally, the preliminary patterns we identified could be valuable in the ongoing search for scientific ideas to enhance the clinical utility of combined strategies for managing the development of cervical cancer.

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APPENDIX A

List of scientific works

Scientific papers published in 2023:

1 Smailova S.B., Shanazarov N.A., Grishacheva T.G., Salmagambetova S.Zh., Aldabergen G.S. Possibilities of fluorescence diagnostics in detecting multicentric focies of cervical dysplasia// BIOMEDICAL PHOTONICS. - 2023.- Vol. 3, No.12. P. 11-14 . DOI: 10.24931/2413–9432–2023–12-3-11–14. (Scopus - 48). (in English)

2 Aitkaliyev A.D., Bekenova N.B., Vochshenkova T.A., Kassiyeva B.S., Alibekov S.R., Shanazarov N.A. «Challenges of false positive cytological results in cervical cancer screening: the future perspective of more reliable testing methods». // Science and Health. 2023. 6 (Vol.25). P. 15-21. DOI 10.34689/SH.2023.25.6.002. (Included in the list of publications recommended SHEQAC MSHE RK). (in English)

3 Shanazarov N.A., Bariyeva G.G., Musin N.M., Albayev R.K., Kaliyev A.A., Iztleuov E.M., Smailova S.B. Photodynamic therapy for cervical cancer: Literature review» // Journal "Oncology and Radiology of Kazakhstan".- 2023.- Vol. 4, No. 70. P.56-63. DOI: 10.52532/2521-6414-2023-4-70-56-63 (included in the list of publications recommended SHEQAC MSHE RK) (in Russian)

Scientific papers published in 2024:

1 Shanazarov NA, Zare A, Mussin NM, Albayev RK, Kaliyev AA, Iztleuov YM, Smailova SB, Tamadon A. Photodynamic therapy of cervical cancer: a scoping review on the efficacy of various molecules // Therapeutic Advances in Chronic Disease. – 2024. – T. 15. – P. 20406223241233206. (Scopus - 79, Web of Science – Q2) (in English)

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3 Shanazarov N.A., Zinchenko S.V., Kisikova S.D., Rizvanov A.A, Smailova S., Petukhov K.A., Salmaganbetova Zh.Zh. Photodynamic therapy in the treatment of HPV-associated cervical cancer: mechanisms, challenges and future prospects // Biomedical Photonics.- 2024.- Vol. 13, No. 1. P. 47–55. doi: 10.24931/2413–9432–2023–13-1-47–55. (Scopus процентиль – 48). (in English)

4 Nasrulla Shanazarov Tatyana Grishacheva Alisher Aitkaliyev, Mbiotech Zhanara Salmaganbetova Sandugash Smailova Balkenzhe Imankulova Maxim Afanasiev Alexander

Dushkin. Assessment of systemic reaction to inflammation induced by photodynamic therapy in cervical intraepithelial neoplasia» // Photodiagnosis and Photodynamic Therapy (Scopus процентиль 86, Web of Science – Q2). (In print). (in English)

5 Shanazarov N.A., Salmaganbetova Zh.Zh., Smailova S.B., Grishacheva T.G. Photodynamic therapy of background and precancerous diseases of the cervix associated with the human papillomavirus : methodological recommendations.- 2024.- Astana .- 33 p. ISBN 978-601-7078-09-6 (in Russian)

6 Shanazarov N.A., Salmaganbetova Zh.Zh., Smailova S.B., Grishacheva T.G. Photodynamic therapy of background and precancerous diseases of the cervix associated with the human papillomavirus : methodological recommendations.- 2024.- Astana .- 33 p. ISBN 978-601-7078-09-6 (in Kazakh)

7 Shanazarov N.A., Grishaeva T.G., Albaev R.K., Zinchenko S.V., Aldabergen G.S. A method of treating background and precancerous diseases of the cervix. Patent for invention No. 36816. Date of receipt: 06/28/2024. The Republic of Kazakhstan. National Institute of Intellectual Property. (in Russian)

8 Shanazarov N.A., Zinchenko S.V., Ismailova S.B., Grishaeva T.G., Kasaeva B.S. Method of treatment of background and precancerous diseases of the cervix. Patent for invention No. 2813949. Date of receipt: 02/20/2024. Russian Federation. Federal Service for Intellectual Property. (in Russian)

9 Shanazarov N. A., Grishacheva T. G., Zinchenkov S. V. photodynamic therapy of HPV-associated diseases of the female genital organs: monograph. - 2024. - Astana. - 168 P. ISBN 978-601-7078-24-9. (in Kazakh)

10 Smailova S.B., Shanazarov N.A., Salmaganbetova Zh.Zh. The first experience of photodynamic therapy in women of reproductive age with HPV-associated cervical dysplasia// Abstracts of the XIV Congress of Oncologists and Radiologists of the CIS and Eurasia. Tajikistan, April 25-27, 2024- T. 12, No. 2, pp. 247 ISSN 2309-7485 (in Russian)

11 Salmaganbetova Zh.Zh., Shanazarov N.A., Smailova S.B., Grishacheva T.G., Zinchenko S.V. Evaluation of the effectiveness of photodynamic therapy in the treatment of HPV-associated squamous intraepithelial lesions of the cervix // Abstracts of the XIV Congress of Oncologists and Radiologists of the CIS and Eurasia. Tajikistan, April 25-27, 2024- T. 12, No. 2, pp. 245. ISSN 2309-7485(in Russian)

12 Abildinova G.Zh., Shanazarov N.A., Voshchenkova T.A., Borovikova A.V., Zhabakova J.M. Candidate genes in patients with HPV-associated cervical dysplasia // Abstracts of the XIV Congress of Oncologists and Radiologists of the CIS and Eurasia. Tajikistan, April 25-27, 2024- T. 12, No. 2, pp. 631. ISSN 2309-7485 (in Russian)

Presentation in 2023

1 Shanazarov N.A. Priority directions of photodynamic therapy in oncology // XII International Congress "Photodynamic therapy and photodiagnostics", Moscow, September 27-28, 2023, p.11(in Russian)

2 Salmaganbetova Zh.Zh., Shanazarov N.A., Grishacheva T.G., Smailova S.B., Aldabergen G.S. An integrated approach to the treatment of cervical cancerous diseases within the framework of the state program for the development of Healthcare of the Republic of Kazakhstan // XII International Congress "Photodynamic therapy and photodiagnostics", Moscow September 27-28, 2023 p.12 (in Russian)

3 N.A. Shanazarov, B.S. Kasieva, T.G. Grishacheva. Comparative analysis of regulatory documents in the field of photodynamic therapy in precancerous diseases of the cervix // XII International Congress "Photodynamic therapy and photodiagnostics", Moscow September 27-28, 2023 p.11 (in Russian)

4 S.B. Smailova, N.A. Shanazarov, T.G. Grishacheva, S.B. Salmagambetova, G.S. Aldabergen. Possibilities of fluorescence diagnostics in the detection of multicentric foci of cervical dysplasia // XII International Congress "Photodynamic therapy and photodiagnostics", Moscow, September 27-28, 2023, p.16 (in Russian)

Presentation in 2024

1 A.E. Hangeldi, M.S. Afanasyev, A.D. Dushkin, T.G. Grishacheva, N.A. Shanazarov, E.S.Biryukova. The effect of photodynamic therapy on Toll-like receptors 2,3,4 and 8 in HPV-associated cervical dysplasia // XIII International Congress "Photodynamic therapy and photodiagnostics", Moscow September 26-27, 2024 p.4 (in Russian)

2 Salmaganbetova Zh.Zh., Shanazarov N.A., Smailova S.B. Effectiveness of combined treatment for HPV-associated cervical dysplasia in the Kazakh population // XIV Congress of Oncologists and radiologists of the CIS and Eurasia, Dushanbe, April 2024 p. 37 (in Russian)

3 Smailova S.B., Shanazarov N.A., Salmagambetova Zh.Zh. The effectiveness of PDT in women with recurrent HPV-associated cervical dysplasia after surgical treatment // XIV Congress of Oncologists and Radiologists of the CIS and Eurasia, Dushanbe, April 2024, p. 37 (in Russian)

APPENDIX B

Patent for invention (Republic of Kazakhstan)

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ РЕСПУБЛИКА КАЗАХСТАН

REPUBLIC OF KAZAKHSTAN

ПАТЕНТ
PATENT

№ 36816

ӨНЕРТАБЫСҚА / НА ИЗОБРЕТЕНИЕ / FOR INVENTION

(21) 2023/0262.1

(22) 13.04.2023

(45) 28.06.2024

(54) Жатыр мойнының фондық және ісік алды ауруларын емдеу тәсілі
Способ лечения фоновых и предраковых заболеваний шейки матки
Method of treatment of background and precancerous cervical diseases

(73) «Қазақстан Республикасы Президентінің Іс Басқармасы Медициналық орталығының ауруханасы» шаруашылық жүргізу құқығындағы республикалық мемлекеттік кәсіпорны (KZ)
Республиканское государственное предприятие «Больница Медицинского центра Управления Делами Президента Республики Казахстан» на праве хозяйственного ведения (KZ)
Republican State EnterpriseMedical Center Hospital of the President's affairs Administration of the Republic of Kazakhstan on the right of economic use (KZ)

(72) Шаназаров Насрулла Абдуллаевич (KZ)
Гришачева Татьяна Георгиевна (RU)
Албаев Рустам Куанышбекович (KZ)
Зинченко Сергей Викторович (RU)
Алдабергенова Гулмира Серікқызы (KZ)

Shanazarov Nasrulla Abdullayevich (KZ)
Grishacheva Tatyana Georgievna (RU)
Albayev Rustam Kuanyshbekovich (KZ)
Zinchenko Sergey Viktorovich (RU)
Aldabergen Gulmira Serikkyzy (KZ)

ЭЦҚ қол қойылды
Подписано ЭЦП
Signed with EDS

Е. Оспанов
Е. Оспанов
Y. Ospanov

«Ұлттық зияткерлік меншік институты» РМК директоры
Директор РГП «Национальный институт интеллектуальной собственности»
Director of RSE «National institute of intellectual property»

APPENDIX C

Patent for invention (Russian Federation)

РОССИЙСКАЯ ФЕДЕРАЦИЯ

(19) **RU** (11) **2 813 949**(13) **C1**

(51) МПК
A61N 5/06 (2006.01)
A61K 31/409 (2006.01)
A61P 35/00 (2006.01)

ФЕДЕРАЛЬНАЯ СЛУЖБА
ПО ИНТЕЛЛЕКТУАЛЬНОЙ СОБСТВЕННОСТИ

(12) ОПИСАНИЕ ИЗОБРЕТЕНИЯ К ПАТЕНТУ

(52) СПК
A61N 5/06 (2024.01); A61K 31/409 (2024.01)

(21)(22) Заявка: **2023123313, 07.09.2023**

(24) Дата начала отсчета срока действия патента:
07.09.2023

Дата регистрации:
20.02.2024

Приоритет(ы):
(22) Дата подачи заявки: **07.09.2023**

(45) Опубликовано: **20.02.2024** Бюл. № **5**

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(56) Список документов, цитированных в отчете о поиске: **ТРИЩЕНКОВА О.В., ЗАРОЧЕНЦЕВА Н.В., ЧУЛКОВА Е.А. Фотодинамическая терапия в лечении предраковых заболеваний шейки матки. Обзор литературы. Вопросы практической кольпоскопии и генитальные инфекции. 2022; (2): 50-54. BY 12759 C1, 30.12.2009. RU 2274478 C1, 20.04.2006. ИЩЕНКО А.И. и др. Фотодинамическая терапия и цервикальная интраэпителиальная** (см. прод.)

RU 2813949 C1

RU 2813949 C1

(54) **Способ лечения фоновых и предраковых заболеваний шейки матки**

(57) Реферат:

Изобретение относится к области медицины, а именно к гинекологии. Проводят внутривенное введение фотосенсибилизатора, затем проводят фотомодификацию крови и флуоресцентную диагностику с последующей фотодинамической терапией шейки матки и цервикального канала. При этом фотосенсибилизатор «Фоторан» или «Фотолон» вводят внутривенно в дозе 1,2 мг/кг массы тела пациента. Через 30 мин проводят фотомодификацию крови на аппарате «Лахта-Милон» с длиной волны 662 нм, выходной мощностью 100 мВт и временем экспозиции 30 минут с помощью магистрального световода «Полироник» и сменных стерильных насадок для внутривенного облучения. Через 3 часа после введения фотосенсибилизатора под контролем флуоресцентной диагностики с помощью светодиодного осветителя с длиной волны 400±15 нм проводят последующее облучение цервикального канала гибкими световодами диффузионного типа с длиной рассеивающей части 4 см, мощностью 500-700 мВт в течение 14-21 мин и наружной порции шейки матки световодами для наружного облучения с мощностью 600-1200 мВт в течение 6-20 мин с плотностью подводимой к очагу энергии 250-400 Дж/см^2. Затем проводят флуоресцентную диагностику с помощью светодиодного

APPENDIX D

Extract from the Minutes of the meeting of the Local Bioethics Commission

Выписка
из Протокола заседания Локальной комиссии по биоэтике
РГП «Больница Медицинского центра Управления делами Президента Республики Казахстан» на ПХВ
№ 2 от «22» октября 2024 года

Число членов Комиссии: 11 человек
Число присутствующих: 10 человек

Повестка дня:

1. Утверждение заключительного отчета исполнителей научно-клинического исследования, проводимого в БМЦ НТП по программно-целевому финансированию: «Разработка инновационных технологий, повышающих эффективность диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных вирусом папилломы человека», научный руководитель д.м.н., профессор Шаназаров Н.А.

Слушали: Авдеев А.В., PhD, руководитель службы аналитической поддержки ТОО «Центр информационных технологий «Даму»» (не аффилированный член ЛКБ).

Результаты голосования (кворум имеется):
«за» - 10.
«против» - 0.
«воздержался» - 0.

Решили: Утвердить заключительный отчет ПЦФ «Разработка инновационных технологий, повышающих эффективность диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных вирусом папилломы человека».

Председатель Комиссии **Абдрахманов А.С.**

Секретарь Комиссии **Касиева Б.С.**

APPENDIX E

Informed consent

ИНФОРМИРОВАННОЕ СОГЛАСИЕ НА УЧАСТИЕ В ИССЛЕДОВАНИИ

Совершеннолетний участник_____
(ФИО)

Исследовательский центр: Больница Медицинского центра Управления Делами Президента Республики Казахстан
Главный исследователь: д.м.н., профессор Шаназаров Насрулла Абдуллаевич
Название исследования: **Разработка инновационных технологий, повышающих эффективность диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных вирусом папилломы человека.**

ИНФОРМАЦИЯ ДЛЯ УЧАСТНИКА ИССЛЕДОВАНИЯ

Мы приглашаем Вас к участию в научном исследовании, проводимому в Больнице Медицинского центра Управления Делами Президента Республики Казахстан г. Астана (далее- Больница).

Мы хотим, чтобы Вы знали:

- Участие в этом исследовании является добровольным.
- Вы можете отказаться от участия в исследовании или выйти из него в любое время. В любом случае вам не будет отказано в том, на что Вы имеете право, не будучи участником исследования.
- Возможно, Ваше участие в исследовании не принесёт Вам дополнительной пользы. Однако в результате исследования мы можем получить знания, которые в будущем принесут пользу другим людям.
- У некоторых людей могут быть личные, религиозные или другие взгляды, которые затрудняют участие в исследовании. Если у Вас есть такие взгляды, пожалуйста, обсудите их со своим врачом или другими специалистами до того, как согласиться на участие.

Прежде чем Вы дадите согласие на участие в исследовании, не спеша, обсудите всё с любым работником данной Больницы или со своими друзьями, родственниками, лечащим врачом или другими специалистами.

Ожидаемая продолжительность участия в исследовании – 24 месяца (04.01.2023-31.12.2024 г.)

Приблизительное (планируемое) количество испытуемых в исследовании: 150 пациентов.

1. НАЗВАНИЕ ИССЛЕДОВАНИЯ:

«Разработка инновационных технологий, повышающих эффективность диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных вирусом папилломы человека»

2. ЦЕЛЬ ИССЛЕДОВАНИЯ

Разработка инновационной технологии, повышающей эффективность диагностики и лечения фоновых и предопухолевых поражений шейки матки, ассоциированных ВПЧ.

3. ОПИСАНИЕ ИССЛЕДОВАНИЯ:

Вам будут проведены следующие методы исследования:

- ✓ гистологическое исследование клеток цервикального канала;

- ✓ цитологическое исследование;
- ✓ фотодинамическая терапия;
- ✓ лабораторное исследование (качественный и количественный ПЦР на ВПЧ);
- ✓ ПЦР диагностика половых инфекций (уреаплазмоз, микоплазмоз, кандидоз);
- ✓ специфические иммунологические методы исследования: CD3, CD4, CD8, CD19, CD16, CD 56; интерлейкин 1, интерлейкин 6, фактор некроза опухоли; (будет производиться забор крови из вены для определения этих показателей).
- ✓ для генетического исследования будет взята кровь из локтевой вены в количестве 2,5 мл.

4. УСЛОВИЯ ОПЛАТЫ/ВОЗМОЖНЫЕ РАСХОДЫ:

За проведение всех диагностических процедур входящих в программу исследования Вы оплачивать не будете. Оплата Вам, как участнику исследования не предусмотрена.

5. ПРЕДСКАЗУЕМЫЕ РИСКИ И НЕУДОБСТВА:

Потенциальные неудобства и риски, связанные с исследованием, не превышают неудобств и рисков при проведении фотодинамической терапии (ФДТ) и введенной физиологической дозы фотосенсибилизатора Фотолон.

Участие в исследовании может потребовать более длительное и периодическое посещение врача, чем обычный прием. Контрольные обследования после фотодинамической терапии будет проведено через 1, 3, 6, 12 мес. Во время проведения процедуры фотодинамической терапии возможен болевой синдром различной степени выраженности (от чувства жжения до резких болей) в зоне облучения, болевые ощущения в зоне воздействия могут сохраняться от нескольких часов до 1 сут., редко отмечается повышение температуры до субфебрильных цифр на 2 сутки после процедуры, что может быть следствием развития асептического воспаления после внутриполостного проведения ФДТ.

Возможны аллергические реакции. В течение 1-2 мес после введения ФС при воздействии солнечного света возможны фототоксические реакции кожи. При повышении температуры до субфебрильных цифр, транзиторном повышении АД специальное лечение не требуется, продолжаются мероприятия в соответствии с разработанной программой. В целом частота осложнений не превышает 5%.

6. ОЖИДАЕМАЯ ПОЛЬЗА:

Впервые будут изучены генетические и иммунологические особенности врожденного иммунного ответа и связанные с ними показатели вирусно-бактериальной нагрузки и оценена эффективность внутривенного лазерного облучения крови для диагностики и лечения фоновых и предопухолевых поражений шейки матки, ассоциированных вирусом папилломы человека (ВПЧ).

Предполагаемые результаты работы могут быть в перспективе широко внедрены в практическое здравоохранение.

7. ПОЛОЖЕНИЕ О ПРАВАХ УЧАСТНИКОВ:

Участие в данном исследовании является добровольным. Вы можете отказаться от участия в исследовании или прекратить участие в любое время. В любом случае Вам не будет отказано в том, на что Вы имеете право, не будучи участником исследования.

8. КОНФИДЕНЦИАЛЬНОСТЬ:

Информация о Вашем участии в исследовании является конфиденциальной. Мы

гарантируем, что Ваше имя не будет указано при публикации результатов исследования. Информация, полученная в результате этого исследования (материалы исследования), считается конфиденциальной и будет храниться в надлежащих условиях, предусмотренных законом. Однако, эти материалы исследования и Ваша личная медицинская документация могут быть доступны для проверок соответствующих подведомственных организаций, в рамках действующих законов или инструкций.

9. КОМПЕНСАЦИЯ/ЛЕЧЕНИЕ:

В случае повреждений, полученных в результате Вашего участия в исследовании, краткосрочная госпитализация и профессиональный уход, если потребуется, будут представлены Вам в РГП «Больница Медицинского центра Управления Делами Президента Республики Казахстан», бесплатно. Финансовая компенсация не предусмотрена. Если Вы полагаете, что получили повреждение, связанное с этим исследованием как участник этого исследования, Вы должны связаться с доктором:

Шаназаров Насрулла Абдуллаевич, по номеру телефона: +7 777 079 1307

10. ЗАВЕРШЕНИЕ УЧАСТИЯ:

Вы можете прекратить участие в исследовании в любое время без каких-либо отрицательных последствий для Вас. Отказ от участия не отразится никоим образом на отношениях к Вам Вашего врача и медицинских работников и Вам не будет отказано в медицинских услугах, на которые Вы имеете право. В случае принятия Вами решения завершить участие раньше предполагаемого срока Вы будете проинформированы о потенциальных последствиях Вашего решения: Вам будет прекращено проведение дополнительных диагностических и лечебных процедур, входящих в программу исследования.

11. КОНТАКТНЫЕ ЛИЦА:

Если у Вас возникают проблемы или вопросы, касающиеся данного исследования, Ваших прав как участника исследования или вреда от исследования, обратитесь к главному исследователю: Шаназарову Насрулле Абдуллаевичу, по номеру телефона: +7 777 079 1307

СОГЛАСИЕ УЧАСТНИКА ИССЛЕДОВАНИЯ

Я прочел (прочла) описание данного исследования.
Мне была представлена возможность обсудить его и задать вопросы.
Настоящим я выражаю свое согласие на участие в данном исследовании.
ФИО участника_____
_____
Подпись участника_____ _____
Дата_____

ФИО исследователя _____
Подпись исследователя_____
Дата_____

APPENDIX F

Calendar plan

Приложение 1.1
к настоящему договору
№ 39-ПИР-23-24 от « 25 » января 2023 года

КАЛЕНДАРНЫЙ ПЛАН

1. Республиканское государственное предприятие «Больница Медицинского центра Управления Делами Президента Республики Казахстан» на праве хозяйственного ведения

1.1 По приоритету: Наука о жизни и здоровье

1.2 По подприоритету: Инновационные исследования в медицине и общественном здравоохранении

1.3 По теме программы: ИРН BR18574160 «Разработка инновационных технологий, повышающих эффективность диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных вирусом папилломы человека».

1.4 Общая сумма программы 400 128 544,00 (четыреста миллионов сто двадцать восемь тысяч пятьсот сорок четыре) тенге 00 тиын, в том числе с разбивкой по годам, для выполнения работ согласно пункту 3:

- на 2023 год - в сумме 225 134 884,00 (двести двадцать пять миллионов сто тридцать четыре тысячи восемьсот восемьдесят четыре) тенге 00 тиын;
- на 2024 год - в сумме 174 993 660,00 (сто семьдесят четыре миллиона девятьсот девяносто три тысяча шестьсот шестьдесят) тенге 00 тиын.

2. Характеристика научно-технической продукции по квалификационным признакам и экономические показатели

2.1 Направление работы: Наука о жизни и здоровье. Инновационные исследования в медицине и общественном здравоохранении.

2.2 Область применения: медицина и здравоохранение.

2.3 Конечный результат:

- за 2023 год. Будет сформирован перечень экзомов для таргентного секвенирования, затем осуществлен набор участников исследования для реализации фотодинамической терапии (далее - ФДТ) у 150 участников исследования с учетом фенотипических данных. Будет сформирована база данных на 200 участников исследования, отображающая клинические особенности, панель биомаркеров, генетические особенности, технические характеристики выполненных методов комплексной технологии. Будут опубликованы 3 статьи или обзора в изданиях, рекомендованных Комитетом по обеспечению качества в сфере образования и науки Министерства науки и высшего образования Республики Казахстан (далее- КОКСОН) и 2 доклада на международных научных форумах.

- за 2024 год. Будет сформирован свод фенотипических и генотипических данных, проведена статистическая обработка данных. Будет проведена интерпретация результатов научного исследования с формированием научного продукта. Будут разработаны новые подходы диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ и возможности персонализированного подхода для профилактики РШМ, ассоциированного с ВПЧ. Будут опубликованы 3 статьи и (или) обзоры в рецензируемых научных изданиях по научному направлению программы, входящих в 1 (первый), 2 (второй), и (или) 3 (третий) квартиль по импакт-фактору в базе данных Web of Science и (или) имеющих процентиль по CiteScore в базе Scopus не менее 50 (пятидесяти),

монография, будет получен патент на изобретение казахстанского патентного бюро и акт внедрения. Изданы методические рекомендации с использованием заключения по независимой научно-медицинской оценке клинического протокола.

2.4 Патентоспособность: Патент на изобретение казахстанского патентного бюро.

2.5 Научно-технический уровень (новизна): Будет проверена гипотеза о возможном кумулятивном эффекте 3 составляющих лазерной технологии с фотосенсибилизацией (ФДТ): флуоресцентная диагностика с применением ФС, оценка «выгорания» ФС при локальной ФДТ и СФВЛОК. Новые научные данные, полученные в ходе проверки гипотезы исследования: генетические и иммунологические особенности врожденного иммунного ответа и связанные с ними показатели вирусно-бактериальной нагрузки, жидкостной цитологии и иммуно-цитохимической оценки содержания p16ink4a будут направлены, исходя из возможной гетерогенности заболевания, на формирование персонализированного, с учетом эндотипа, подхода в выборе ФДТ.

2.6 Использование научно-технической продукции осуществляется: пациент женского пола казахского этноса старше 18 лет.

2.7 Вид использования результата научной и (или) научно-технической деятельности: Прикладное.

3. Наименование работ, сроки их реализации и результаты

Шифр задания, этапа	Наименование работ по Договору и основные этапы его выполнения*	Срок выполнения*		Ожидаемый результат*
		начало	окончание	
	2023 год			
1.	Оценка эффективности и безопасности применяемых методов лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ.	январь	март	Обзор международных профильных исследований для оценки аргументов в пользу научной основы исследования и формирования перечня экзомов для таргетного секвенирования.
1.1.	Обзор международных профильных исследований, их сравнительный анализ с ФДТ, детальное рассмотрение механизмов биологических эффектов ФДТ.	январь	март	Перечень аргументов для научной основы разрабатываемой технологии, согласно цели и задачам исследования.
1.2.	Формирование перечня экзомов для таргетного секвенирования.	январь	март	Перечень экзомов для таргетного секвенирования.
2.	Разработка усовершенствованного, эффективного и безопасного метода лечения фоновых и предопухолевых заболеваний	январь	До 1 ноября	Реализация комплексной технологии ФДТ у 200 участников исследования с

	шейки матки, ассоциированных с ВПЧ, с применением лазерных технологий с фотосенсибилизацией (ФДТ).			учетом фенотипических данных. Формирование сводной базы данных у 200 участников исследования.
2.1.	Выполнение ФДТ участникам исследования.	январь	До 1 ноября	Реализация ФДТ у 150 участников исследования.
2.2.	Формирование базы данных о выполненной ФДТ.	январь	До 1 ноября	База данных о выполненной ФДТ на 150 участников исследования.
3.	Разработка метода флуоресцентной диагностики с применением ФС при фоновых и предопухолевых заболеваниях шейки матки, ассоциированных ВПЧ.	январь	До 1 ноября	Реализация метода у 150 участников исследования с учетом фенотипических данных Формирование базы данных у 150 участников исследования.
3.1.	Выполнение метода участниках исследования, последующее наблюдение 3, 6, 9, 12 месяцев.	январь	До 1 ноября	Реализация метода у 150 участников исследования.
3.2.	Формирование базы данных о выполненном методе.	январь	До 1 ноября	База данных о выполненном методе у участников исследования.
4.	Разработка метода оценки «выгорания» ФС при проведении локальной ФДТ.	январь	До 1 ноября	Реализация метода у 150 участников исследования с учетом фенотипических данных. Формирование базы данных у 150 участников исследования.
4.1.	Выполнение метода участникам исследования, последующее наблюдение 3, 6, 9, 12 месяцев.	январь	До 1 ноября	Выполнение метода у 150 участников исследования
4.2.	Формирование базы данных о выполненном методе.	январь	До 1 ноября	База данных о выполненном методе у 150 участников исследования.
5.	Разработка и внедрение оптимальной программы проведения ФДТ для полной эрадикации ВПЧ.	январь	До 1 ноября	Проведение комплексной программы ФДТ у 150 участников исследования. Формирование научного продукта исследования.
5.1.	Рекрутинг и идентификация участников исследования.	январь	До 1 ноября	Включение в исследование участников.

5.2.	Проведение таргетного экзомного секвенирования ДНК участников исследования.	январь	До 1 ноября	База данных об исследуемом генетическом статусе 200 участников исследования.
5.3.	Проведение оценки иммунологического статуса участников исследования.	январь	До 1 ноября	База данных о иммунологическом статусе 200 участников исследования. Будет принята / опубликована 1 статья или обзор в издании, рекомендованном КОКСОН. 2 доклада на международных научных форумах.
5.4.	Проведение оценки клинического статуса участников исследования.	январь	До 1 ноября	База данных о клиническом статусе участников исследования.
5.5.	Проведение исследования распространенности развития фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ.	январь	До 1 ноября	Отчет. Будет принята / опубликована 1 статья или обзор в издании, рекомендованном КОКСОН.
5.6.	Формирование базы данных по участникам исследования.	январь	До 1 ноября	База данных на 200 участников исследования, отображающая клинические особенности, панель биомаркеров, генетические особенности, технические характеристики выполненных методов комплексной технологии. Будет принята / опубликована 1 статья или обзор в издании, рекомендованном КОКСОН.
5.7.	Обеспечение осведомленности населения о факторах риска развития и методах лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных ВПЧ,	январь	До 1 ноября	Веб-сайт с краткой информацией о реализации научно-технической программы.

	проводимом исследовании и его результатах.			
	2024 год			
2.	Разработка усовершенствованного, эффективного и безопасного метода лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ, с применением лазерных технологий с фотосенсибилизацией (ФДТ).	январь	До 1 ноября	Инновационная комплексная эффективная технология диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ. Заключение по гипотезе исследования, изложенное в заявленных научных продуктах.
2.1.	Формирование базы данных в 3, 6, 9, 12 месяцев наблюдения за участниками исследования.	январь	июль	База данных об участниках исследования.
2.2.	Анализ результатов использования технологии в ассоциации с клиническими, иммунологическими и генетическими данными.	июль	октябрь	Аналитическая справка по результатам использования технологии в ассоциации с клиническими, иммунологическими и генетическими данными.
2.3.	Формирование заключения по оптимальной программе выполнения разработанной технологии для целевой группы пациенток Формирование заключения по технологии ФДТ для целевой группы пациенток.	июль	октябрь	Обоснованные выводы и рекомендации по новому подходу к лечению фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ.
3.	Разработка метода флуоресцентной диагностики с применением ФС при фоновых и предопухолевых заболеваниях шейки матки, ассоциированных ВПЧ.	январь	До 1 ноября	Формирование базы данных 200 участников исследования. Заключение по эффективности используемого метода гипотезе исследования.
3.1.	Формирование базы данных.	январь	Июль	База данных об участниках исследования.
3.2.	Анализ результатов использования технологии в ассоциации с иммунологическими и	июль	Октябрь	Заключение по эффективности используемого метода гипотезе исследования.

	генетическими данными.			
3.3.	Формирование заключения по методу для целевой группы пациенток.	июль	октябрь	Обоснованные выводы и рекомендации по методу.
4.	Разработка метода оценки «выгорания» ФС при проведении локальной ФДТ. Данный метод, входящий в основную технологию, будет оценен с точки зрения эффективности различных модификаций, будут сформированы обоснованные выводы и рекомендации.	январь	До 1 ноября	Формирование базы данных 200 участников исследования. Заключение по эффективности используемого метода гипотезе исследования.
4.1.	Формирование базы данных.	январь	июль	Рекрутинг и идентификация 200 участников исследования, последующее наблюдение за 200 участниками исследования.
4.2.	Анализ результатов использования технологии в ассоциации с иммунологическими и генетическими данными.	июль	До 1 ноября	Заключение по эффективности используемого метода гипотезе исследования.
4.3.	Формирование заключения по методу для целевой группы пациенток.	июль	октябрь	Обоснованные выводы и рекомендации по методу.
5.	Разработка и внедрение оптимальной программы проведения ФДТ для полной эрадикации ВПЧ.	январь	До 1 ноября	Заключение по гипотезе исследования. Формирование научного продукта по разработанной комплексной технологии.
5.1.	Свод, систематизация и изучение собранных и полученных данных, формирование научной основы для интерпретации результатов исследования.	январь	июль	Свод фенотипических и генотипических данных, статистическая обработка данных.
5.2.	Интерпретация результатов исследования с формированием научного продукта.	январь	До 1 ноября	Новые подходы диагностики и лечения фоновых и предопухолевых

				заболеваний шейки матки, ассоциированных с ВПЧ. Возможности персонализированного подхода для профилактики РШМ, ассоциированного с ВПЧ. 3 статьи и (или) обзоры в рецензируемых научных изданиях по научному направлению программы, входящих в 1 (первый), 2 (второй), и (или) 3 (третий) квартиль по импакт-фактору в базе данных Web of Science и (или) имеющих процентиль по CiteScore в базе Scopus не менее 50 (пятидесяти). Монография
5.3.	Внедрение комплексной технологии для диагностики и лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных с ВПЧ ведения женщин с заболеваниями шейки матки, ассоциированными с ВПЧ, с использованием персонализированного подхода.	январь	До 1 ноября	Патент на изобретение казахстанского патентного бюро. Акт внедрения.
5.4.	Внесение предложений по пересмотру клинических протоколов диагностики и лечения.	январь	До 1 ноября	Методические рекомендации с использованием заключения по независимой научно-медицинской оценке клинического протокола.
5.5.	Обеспечение осведомленности населения о факторах риска развития и методах лечения фоновых и предопухолевых заболеваний шейки матки, ассоциированных ВПЧ, проводимом исследовании и его	январь	До 1 ноября	Веб-сайт с краткой информацией.

	результатах.			
5.6.	Подготовка итогового отчета по научно-технической программе.	сентябрь	До 1 ноября	Отчет, принятый НЦГНТЭ.

От Заказчика:
Заместитель председателя
ГУ «Комитет науки Министерства науки и высшего образования РК».

Орынбеков Д.Р.

От Исполнителя:
Директор
РГП «Больница Медицинского центра Управления Делами Президента Республики Казахстан» на ПХВ

Албаев Р.К.

Научный руководитель программы

Шаяхзаров Н.А.
(подпись)

1. Republican state enterprise “Medical Center Hospital of the President’s Affairs Administration of the Republic of Kazakhstan” with the right of economic management

1.1 Priority: The program's top priority is "Science of Life and Health."

1.2 Sub-priority: The secondary priority is "Innovative Research in Medicine and Public Health."

1.3 Program Theme: The program's theme is identified as "IRN BR18574160 - The development of innovative technologies increasing diagnosis and treatment effectiveness of the cervix background and precancerous diseases associated with HPV”.

1.4 Total Program Budget: The overall budget for this program is 400,128,544.00 (four hundred million, one hundred twenty-eight thousand, five hundred forty-four) Kazakhstani tenge, with a breakdown by year:

- For the year 2023: 225,134,884.00 (two hundred twenty-five million, one hundred thirty-four thousand, eight hundred eighty-four) Kazakhstani tenge.
- For the year 2024: 174,993,660.00 (one hundred seventy-four million, nine hundred ninety-three thousand, six hundred sixty) Kazakhstani tenge.

2. Characteristics of scientific and technical products according to qualification criteria and economic indicators

2.1 Work Direction: Life and Health Sciences. Innovative research in medicine and public health.

2.2 Application Area: Medicine and healthcare.

2.3 Final Outcome:

- In 2023, a list of exomes for targeted sequencing will be established, followed by the recruitment of research participants for the implementation of photodynamic therapy (hereinafter referred to as PDT) in 150 research participants, taking into account phenotypic data. A database will be created for 200 research participants, reflecting clinical characteristics, a panel of biomarkers, genetic features, and technical specifications of the methods used in the comprehensive technology. Three articles or reviews will be published in publications recommended by the list of journals recommended by the committee for quality assurance of education and science of the MES of the Republic of Kazakhstan (hereinafter referred to as COCSON) and two presentations at international scientific forums.
- In 2024, a compilation of phenotypic and genotypic data will be generated, and statistical data processing will be conducted. The results of the scientific research will be interpreted to create a scientific product. New approaches to the diagnosis and treatment of cervical background and precancerous diseases associated with HPV will be developed, along with the potential for a personalized approach to prevent cervical cancer associated with HPV. Three articles and/or reviews will be published in peer-reviewed scientific publications in the field of the program's scientific direction, which fall within the 1st, 2nd, and/or 3rd quartile based on impact factor in the Web of Science database and/or have a CiteScore percentile in Scopus of no less than 50 (fifty). A monograph will be published, a patent will be obtained from the Kazakhstan Patent Office, and an implementation report will be issued. Methodological recommendations will be published using the conclusion of an independent scientific and medical assessment of the clinical protocol.

2.4 Patentability: Patent from the Kazakhstan Patent Office.

2.5 Scientific and Technical Level (Novelty): The hypothesis regarding the possible cumulative effect of three components of laser technology with photosensitization (PDT) will be tested: fluorescent diagnostics using PS, evaluation of PS "burnout" during local PDT, and SFVLOK. New scientific data obtained during the testing of the research hypothesis will focus on genetic and immunological features of the innate immune response and related indicators of viral-bacterial load, liquid cytology, and immunocytochemical assessment of p16ink4a content. These data will be used, considering the potential disease heterogeneity, to develop a personalized approach for selecting PDT based on endotype.

2.6 Utilization of scientific and technical products is carried out by female patients of Kazakh ethnicity aged 18 and older.

2.7 Type of use of the results of scientific and/or scientific-technical activities: Applied.

3. Name of work, timing of their implementation and results

Code of task, stage	Name of work under the Contract and the main stages of its implementation*	Deadline*		Expected results*
		start	finish	
2023				
1.	Assessment of the effectiveness and safety of the applied methods of treatment of precancerous diseases of the cervix associated with HPV.	January	March	A review of international specialized studies to evaluate the arguments in favor of the scientific basis of the study and the creation of a list of exomes for targeted sequencing.
1.1.	Review of international specialized studies, their comparative analysis with PDT, detailed consideration of the mechanisms of biological effects of PDT.	January	March	A list of arguments for the scientific basis of the technology being developed, according to the purpose and objectives of the research.
1.2.	Formation of a list of exomes for targeted sequencing.	January	March	List of exomes for targeted sequencing.
2.	Development of an improved, effective and safe method for the treatment of background and precancerous diseases of the cervix associated with HPV using laser technologies with photosensitivity (PDT).	January	Until November 1	Implementation of complex PDT technology in 200 study participants, taking into account phenotypic data. Formation of a consolidated database for 200 study participants.
2.1.	Performing PDT on study participants.	January	Until November 1	Implementation of PDT in 150 study participants.
2.2.	Formation of a database of performed PDT.	January	Until November 1	Database of PDT performed for 150 study participants.
3.	Development of a fluorescent diagnostic method using PDT for background and precancerous diseases of the cervix associated with HPV.	January	Until November 1	Implementation of the method in 150 study participants, taking into account phenotypic data Formation of a database for 150 study participants.

3.1.	Implementation of the method for study participants, follow-up for 3, 6, 9, 12 months.	January	Until November 1	Implementation of the method among 150 study participants.
3.2.	Formation of a database about the performed method.	January	Until November 1	Database on the method performed by study participants.
4.	Development of a method for assessing the "burnout" of the FS during local PDT.	January	Until November 1	Implementation of the method in 150 study participants, taking into account phenotypic data.
4.1.	Implementation of the method for study participants, follow-up for 3, 6, 9, 12 months.	January	Until November 1	Formation of a database for 150 study participants.
4.2.	Formation of a database about the performed method.	January	Until November 1	Database on the method performed on 150 study participants.
5.	Development and implementation of an optimal PDT program for complete eradication of HPV.	January	Until November 1	Conducting a comprehensive PDT program for 150 study participants.
5.1.	Recruitment and identification of study participants.	January	Until November 1	Formation of a scientific research product.
5.2.	Conducting terget exome DNA sequencing of study participants.	January	Until November 1	Database on the studied genetic status of 200 study participants.
5.3.	Assessing the immunological status of study participants.	January	Until November 1	Database on the immunological status of 200 study participants. 1 article or review will be accepted/published in a publication recommended by COCSON. 2 reports at international scientific forums.
5.4.	Assessing the clinical status of study participants.	January	Until November 1	Database on the clinical status of study participants.
5.5.	Conducting a study of the prevalence of the development of background and pre-tumor diseases of the cervix associated with HPV.	January	Until November 1	Report. 1 article or review will be accepted/published in a publication recommended by COCSON.
5.6.	Formation of a database of study participants.	January	Until November 1	Database for 200 study participants, displaying clinical features, a panel of biomarkers, genetic features, technical characteristics of the complex technology

				methods performed. 1 article or review will be accepted/published in a publication recommended by COCSON.
5.7.	Ensuring public awareness of risk factors for the development and methods of treatment of background and precancerous diseases of the cervix associated with HPV, the ongoing research and its results.	January	Until November 1	Website with brief information about the implementation of the scientific and technical program.
2024				
2.	Development of an improved, effective and safe method for the treatment of background and precancerous diseases of the cervix associated with HPV using laser technologies with photosensitivity (PDT).	January	Until November 1	Innovative comprehensive effective technology for the diagnosis and treatment of background and precancerous diseases of the cervix associated with HPV. Conclusion on the research hypothesis set out in the claimed scientific products.
2.1.	Formation of a database at 3, 6, 9, 12 months of observation of study participants.	January	July	Database of study participants.
2.2.	Analysis of the results of using technology in association with clinical, immunological and genetic data.	July	October	Analytical report on the results of using technology in association with clinical, immunological and genetic data.
2.3.	Formation of a conclusion on the optimal program for implementing the developed technology for the target group of patients Formation of a conclusion on PDT technology for the target group of patients.	July	October	Substantiated conclusions and recommendations for a new approach to the treatment of background and precancerous cervical diseases associated with HPV.
3.	Development of a fluorescent diagnostic method using PS for background and precancerous diseases of the cervix associated with HPV.	January	Until November 1	Formation of a database of 200 study participants. Conclusion on the effectiveness of the method used and the research hypothesis.

3.1.	Formation of the database.	January	July	Database of study participants.
3.2.	Analysis of the results of using technology in association with immunological and genetic data.	July	October	Conclusion on the effectiveness of the method used and the research hypothesis.
3.3.	Formation of a conclusion on the method for the target group of patients.	July	October	Substantiated conclusions and recommendations for the method.
4.	Development of a method for assessing the "burnout" of the FS during local PDT. This method, which is part of the core technology, will be assessed from the point of view of the effectiveness of various modifications, and reasonable conclusions and recommendations will be formed.	January	Until November 1	Formation of a database of 200 study participants. Conclusion on the effectiveness of the method used and the research hypothesis.
4.1.	Formation of the database.	January	July	Recruitment and identification of 200 study participants, follow-up of 200 study participants.
4.2.	Analysis of the results of using technology in association with immunological and genetic data.	July	Until November 1	Conclusion on the effectiveness of the method used and the research hypothesis.
4.3.	Formation of a conclusion on the method for the target group of patients.	July	Until November 1	Substantiated conclusions and recommendations for the method.
5.	Development and implementation of an optimal PDT program for complete eradication of HPV.	January	Until November 1	Conclusion on the research hypothesis. Formation of a scientific product using the developed complex technology.
5.1.	Compilation, systematization and study of collected and received data, formation of a scientific basis for interpreting research results.	January	Until November 1	Collection of phenotypic and genotypic data, statistical data processing.
5.2.	Interpretation of research results with the formation of a scientific product.	January	Until November 1	New approaches to the diagnosis and treatment of background and precancerous diseases of the cervix associated with HPV.

				Possibilities of a personalized approach for the prevention of HPV-associated cervical cancer. 3 articles and (or) reviews in peer-reviewed scientific publications in the scientific area of the program, included in the 1st (first), 2nd (second), and (or) 3rd (third) quartile by impact factor in the Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 50 (fifty). Monograph
5.3.	Introduction of complex technology for the diagnosis and treatment of background and precancerous cervical diseases associated with HPV and management of women with HPV-associated cervical diseases using a personalized approach.	January	Until November 1	Patent for an invention from the Kazakhstan Patent Bureau. Act of implementation.
5.4.	Making proposals for revising clinical protocols for diagnosis and treatment.	January	Until November 1	Methodological recommendations using the conclusion of an independent scientific and medical assessment of the clinical protocol.
5.5.	Ensuring public awareness of risk factors for the development and methods of treatment of background and precancerous diseases of the cervix associated with HPV, the ongoing research and its results.	January	Until November 1	Website with brief information.
5.6.	Preparation of the final report on the scientific and technical program.	сентябрь	Until November 1	Report accepted by NCSTE