ANNOTATION

of the dissertation by Khangeldi Akmaral Yeskeldikyzy on the topic "Changes in Immune Status during Photodynamic Therapy of HPV-Associated Precancerous Diseases of the Cervix in Women of Reproductive Age of the Kazakh Population" submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D10102 – "Medicine"

Relevance of the Topic Cervical cancer (CC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related mortality among women worldwide. In 2020, the International Agency for Research on Cancer (IARC) reported 603,863 newly diagnosed cases of cervical cancer and 341,680 deaths attributable to the disease.

In the Republic of Kazakhstan, cervical cancer remains the most common oncological pathology among women aged 15–44 years. According to data from the International Agency for Research on Cancer and the ICO/IARC Information Centre on HPV and Cancer, more than 1,777 women are diagnosed with cervical cancer annually, and 834 die from this disease. Cervical cancer accounts for 7.7% of all cancer-related deaths among women.

The National Cancer Registry of the Republic of Kazakhstan provides comprehensive information on incidence trends. According to statistics for 2023, 1,921 new cases of cervical cancer were registered, accounting for 7.2% of all female oncological diseases. The mean age of affected patients ranges from 45 to 49 years, indicating involvement of the most socially and economically active segment of the female population. Of particular concern is the fact that the majority of cases are diagnosed at advanced stages, when treatment effectiveness is significantly reduced and prognosis worsens.

In order to improve the effectiveness of treatment for patients with HPV-associated cervical diseases, combined therapeutic approaches using laser technologies have been proposed. These approaches include local photodynamic therapy (PDT) in combination with systemic intravenous laser blood irradiation. PDT is a non-destructive method for the treatment of cervical dysplasia that combines the application of a photosensitizer to affected tissues with exposure to a specific light source that activates the photosensitizer, leading to selective destruction of abnormal cells.

Aim of the Study

To evaluate the characteristics of immune status in women with HPV-associated cervical dysplasia during photodynamic therapy.

Objectives of the Study

1. To determine and compare the expression of TLR2, TLR3, TLR4, and TLR8 mRNA in exocervical smear scrapings in patients with cervical HPV infection presenting with different grades of squamous intraepithelial lesions.
2. To assess the effectiveness of photodynamic therapy (PDT) in patients with HPV infection according to virological and cytological criteria depending on the initial grade of cervical squamous intraepithelial lesion.

3. To determine the dynamics of TLR2, TLR3, TLR4, and TLR8 mRNA expression in exocervical smear scrapings after photodynamic therapy.

Object and Subject of the Study

A randomized controlled study was conducted. The study included 170 women of reproductive age of the Kazakh population diagnosed with HPV-associated precancerous cervical diseases. The main group consisted of 120 patients with morphologically confirmed cervical dysplasia:LSIL — 54 patients, HSIL — 66 patients, the control group included 50 women without cervical pathology. The study was carried out during the period from 2023 to 2024 at the clinical base of the Department of Obstetrics and Gynecology No. 1 of Astana Medical University at the Hospital of the Medical Center of the Administrative Office of the President of the Republic of Kazakhstan. Patients in the main group underwent photodynamic therapy in accordance with the approved clinical protocol. Written informed consent was obtained from all participants prior to inclusion in the study.

Research Methods

1. Clinical methods (collection of clinical and anamnestic data).
2. Molecular genetic methods (assessment of TLR2, TLR3, TLR4, and TLR8 mRNA expression using polymerase chain reaction (PCR)).
3. Intervention method (photodynamic therapy including intravenous administration of the photosensitizer “Photoran” 180–200 minutes prior to the procedure; fluorescence cervical diagnostics; laser irradiation of the cervix).
4. Instrumental methods (extended colposcopy; cervical biopsy with morphological examination).
5. Laboratory methods (HPV typing and viral load determination using PCR; cytological examination; cytokine level determination; complete blood count).
6. Statistical methods (statistical processing and analysis of the obtained data).

Scientific Novelty

1. For the first time, the expression of TLR2, TLR3, TLR4, and TLR8 mRNA in cervical squamous epithelium in patients with HPV-associated precancerous cervical disease was studied before and after photodynamic therapy *in vivo* in the Kazakh population.
2. A novel photodynamic therapy protocol aimed at HPV eradication and prevention of malignant transformation in cervical dysplasia was developed for the first time.
3. The dependence of PDT effectiveness on the baseline level of TLR mRNA expression and the dynamics of its changes after treatment was demonstrated for the first time.
4. The developed comprehensive assessment of immune status (including the dynamics of TLR mRNA expression) during photodynamic therapy for HPV-associated precancerous cervical diseases in women of reproductive age has been registered as an object of copyright in the Republic of Kazakhstan (Certificate No. 67021 dated 05.01.2026). The developed approaches to photodynamic therapy for background and precancerous diseases of the female genital organs are protected by Patent of the Russian Federation for invention No. 2840195 dated 19.05.2025.

Practical Significance

1. The developed and clinically tested photodynamic therapy protocol using the photosensitizer “Photoran” (chlorin e6) and the “Lakhta-Milon” device ensures eradication of high-risk HPV in 93.3% of cases in the LSIL group and in 84.5% of cases in the HSIL group three months after treatment ($p=0.002$ and $p<0.001$, respectively), as well as cytological regression of SIL in 93.3–94.9% of cases (n=120). This makes PDT a promising non-invasive alternative to surgical treatment methods (conization, loop excision) in reproductive-aged women, in whom repeated surgical interventions may lead to cervical shortening, cervical insufficiency, and an increased risk of preterm birth.

2. The identified differences in TLR2 and TLR4 mRNA expression, as well as in the degree of immune cell infiltration of the exocervix (pronounced infiltration in 58.3% of HSIL cases versus 0% in LSIL, $p=0.001$) and the number of leukocytes in cervical secretion (median 13 in HSIL versus median 5 in LSIL, $p<0.001$), may be used in clinical practice as additional molecular-immunological criteria for stratifying the risk of dysplasia progression and selecting treatment strategies.

3. Early post-procedural dynamics of mucosal immunity markers (a 1.5-fold increase in IFN-γ concentration two hours after PDT, $p=0.047$; a 13% decrease in TLR2 mRNA expression; and a 16.7% increase in TLR4 mRNA expression) allow PDT to be considered a method with a predictable immunomodulatory effect, opening prospects for early monitoring of therapeutic response.

4. The obtained data on 12-month recurrence-free survival (77.8% HPV-free survival and 89.4% SIL-free survival in the LSIL group; 80.3% HPV-free survival and 94.9% SIL-free survival in the HSIL group) justify consideration of PDT as an additional or alternative method in the comprehensive treatment of HPV-associated precancerous cervical conditions.

5. The results of the study have been implemented in the practical activities of the Hospital of the Medical Center of the Administrative Office of the President of the Republic of Kazakhstan. A patent for invention and a copyright certificate for the method of using PDT in HPV-associated diseases have been obtained.

Main Provisions Submitted for Defense

1. The severity of mucosal immunity and the expression of TLR2 and TLR4 mRNA significantly differ depending on the grade of dysplasia.

2. The developed PDT protocol provides high efficacy of high-risk HPV eradication and cytological regression of SIL.

3. Photodynamic therapy induces early immune activation and provides a sustained recurrence-free period.

Conclusions

1. In reproductive-aged women with HPV-associated cervical diseases, progressive suppression of local immune response is observed with increasing severity of dysplasia.

2. Three months after PDT, a significant decrease in HPV-positive patients is observed, confirming high therapeutic efficacy.

3. Early immune activation after PDT is associated with local inflammatory response and prolonged recurrence-free survival.

4. The results expand current understanding of TLR mRNA in the pathogenesis of HPV-associated cervical lesions and substantiate the use of PDT as an effective immunomodulatory treatment.

Practical Recommendations

The method may be recommended as an optimal non-invasive treatment for HPV-associated cervical dysplasia in women of reproductive age. Implementation of this algorithm in gynecological departments and screening programs in the Republic of Kazakhstan will allow personalization of therapeutic approaches, reduction of surgical complications, and improvement of reproductive outcomes. Further research is required for inclusion of PDT in national clinical guidelines.

Structure and Scope of the Dissertation

The dissertation comprises 126 pages and includes an introduction, literature review, materials and methods, results, discussion, conclusions, references, practical recommendations, and appendices. The work contains 8 tables and 32 figures. The reference list includes 201 sources.