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EVALUATION OF THE EFFECTIVENESS OF MODERN TREATMENT APPROACHES FOR PRECANCEROUS DISEASES OF THE VULVA

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Abstract

The aim of the study was to evaluate the effectiveness of modern approaches to the treatment of patients with various types of vulvar intraepithelial neoplasia. **Materials and methods.** A prospective study of the results of VIN treatment in 262 women aged 25 to 70 years (mean age 51.12±13.41 years) was conducted. Treatment was provided to 155 women with HPV-independent vulvar dysplasia (dVIN), 98 women with severe HPV-independent vulvar dysplasia (VHSIL), and 9 women with grade Ia vulvar Paget's disease. Depending on the type and features of the disease, clinical and morphological characteristics, size, location, age and anamnesis, a particular treatment method was chosen, namely: excision, photodynamic therapy (PDT) with chlorine E6; PDT with 5-aminolevulinic acid; 5% imiquimod cream; vulvectomy (partial/complete). In some cases, a combination of methods was used: excision and 5% imiquimod cream; excision with PDT with 5-aminolevulinic acid. **Results.** Cream with 5% imiquimod was used in our study mainly in women with VHSIL and showed quite high efficacy in the treatment process. After 3 months, partial regression was registered in 93.75% of cases, after six months, complete regression occurred in 71.87% of patients, and after a year - in 81.25% of women. Morphologically, after 3 months, the recurrence of the

disease was registered only in 31.25% of patients, while over time a complete coincidence of clinical and morphological indicators was registered. Combined therapy with imiquimod showed high efficacy both clinically and morphologically with complete regression of the disease (100%) and absence of relapses during the year of follow-up in all patients to whom this approach was used. Treatment with the systemic use of PDT with chlorin E6 showed a complete regression of the pathological focus clinically and morphologically in 96.65% of women after 3 months. The same indicators remained after 6 months, but after a year, the percentage of women with partial regression with morphologically confirmed recurrence slightly increased to 15.08%. **Conclusions.** Modern methods of conservative treatment of vulvar intraepithelial neoplasia using imiquimod cream and systemic photodynamic therapy can be a highly effective alternative to surgical intervention.

Keywords: vulvar intraepithelial neoplasia; vulvectomy; photodynamic therapy; treatment of vulvar intraepithelial neoplasia.

According to the WHO 2020 classification, precancerous diseases of the vulva include high-grade HPV-dependent vulvar squamous intraepithelial neoplasia (VHSIL), differentiated vulvar intraepithelial dysplasia (dVIN), and grade Ia Paget's disease of the vulva [1]. These diseases have received attention in recent years for two reasons. Firstly, they have a fairly high risk of malignancy: 32.8% for dVIN, up to 19% for Paget's disease, and 5.7% for VHSIL [2-5]. Second, according to large international studies, the incidence of vulvar intraepithelial neoplasia is increasing, especially in young women [6-8]. In the past, surgery was the standard of care to remove the pathological lesion. But it is significantly traumatic both physically and mentally. Therefore, the current goals are now to prevent the progression of squamous cell carcinoma of the vulva, preserve normal anatomy, relieve symptoms, maintain quality of life and sexual function through individualised treatment [9]. Currently, a large number of surgical and medical treatments for vulvar intraepithelial neoplasia (VIN) have been proposed, but data on their effectiveness vary considerably according to different authors [9].

Thus, **the aim of the study** was to evaluate the effectiveness of modern approaches to the treatment of patients with various types of vulvar intraepithelial neoplasia.

Research materials and methods

To solve this problem, we prospectively studied the results of VIN treatment in 262 women aged 25 to 70 years (mean age 51.12±13.41 years) who needed medical care at the National Cancer Institute (Kyiv, Ukraine) in 2017-2023. Patients were enrolled in the study

after obtaining written informed consent (describing the planned treatment and diagnostic procedures, possible benefits and risks, adverse reactions and complications, timing of follow-up visits and compliance with post-treatment recommendations) in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and relevant laws of Ukraine. The diagnosis was made on the basis of medical history, complaints, clinical examination, vulvoscopy and the results of morphological examination of pathologically altered tissues. The criteria for including patients in the study for treatment were histological confirmation of the diagnosis, absence of severe comorbidities, and written consent to treatment.

Treatment was provided to 155 women with HPV-independent vulvar dysplasia (dVIN), 98 women with severe HPV-independent vulvar dysplasia (VHSIL), and 9 women with grade Ia Paget's disease of the vulva (adenocarcinoma in situ of the vulvar skin).

Depending on the type and features of the disease, clinical and morphological characteristics, size, location, age and anamnesis, a particular treatment method was chosen, namely: excision, photodynamic therapy (PDT) with chlorine E6; PDT with 5-aminolevulinic acid; 5% imiquimod cream; vulvectomy (partial/complete). In some cases, a combination of methods was used: excision and 5% imiquimod cream; excision with PDT with 5-aminolevulinic acid.

For PDT, the agent "Photolon®" was used as a photosensitizer (PS), which is a complex of trisodium salt of chlorine E6 with low-molecular weight polyvinylpyrrolidone. PS was dissolved in 100 ml of physiological saline and administered intravenously over 30 minutes in doses ranging from 1 to 2.5 mg/kg of patient body weight in a darkened room. The PDT session was performed 3-4 hours after the end of the PS infusion using a universal laser coagulator "Lika-Surgeon" (Photonics Plus, Ukraine, λ = 660 nm). The size of the photoirradiation fields varied from 1 to 4 cm, the number of fields - from 1 to 5, the radiation power - 0.4-1 W, the exposure dose of light - from 100 to 150 J/cm2. The duration of the session depended on the degree of pathological foci prevalence and ranged from 5-90 minutes, depending on the number of irradiation fields. Normal vulvar tissues were included in the photoirradiation zone with a distance of at least 5 mm from the border of the lesion. Due to the high sensitivity of the photoirradiation zone, premedication with non-narcotic analgesic drugs (ketorolac tromethamine 30 mg) was performed 30 minutes before the PDT session to relieve pain.

In some cases, photodynamic therapy with topical application of PS in the form of 6% 5-aminolevulinic acid phosphate gel (Levuderm) was used. After treating the vulvar surface

with saline, before applying the photosensitizer, the surface crusts or scales in the treatment area were carefully scraped off to ensure better penetration of the PS into the tissues. Subsequently, a 6% 5-aminolevulinic acid phosphate gel (Levuderm) was applied to the lesion and approximately 5 mm of the surrounding area with a thickness of approximately 1 mm. The treatment area was then covered with an occlusive dressing for 2 hours. After this time, the dressing was taken off and the excess photosensitizer was removed before irradiation. The irradiation was performed with the TrevioLux irradiation system (from MEDlight) with a spectral range of LEDs of about 630 nm. The energy applied to the lesion was on average 30-40 J/cm2. The light power density was 0.1-1.0 W/cm2, and the exposure time varied depending on the lesion area. An exposure distance of 15 cm was maintained throughout the entire irradiation session. Phototherapy was performed once every 2 weeks, the duration of the sessions was 10-15 minutes, the number of sessions was 4-6, depending on the area of the lesion, closure of tissue defects (cracks, erosion).

Patients applied the cream with 5% imiquimod on their own three times a week (e.g. on Monday, Wednesday and Friday or Tuesday, Thursday and Saturday) in a thin layer and rubbed it into a clean surface of the affected areas until it was completely absorbed before going to bed and left on the skin for 6-10 hours, avoiding showering or bathing. The cream was applied only to the affected areas. After the specified period, the cream was washed off with warm water and mild soap. One sachet of cream is sufficient for application to a 20 cm2 area of skin with a lesion. Patients were not allowed to reuse the cream from a previously opened sachet. Before and after applying the cream, the women washed their hands thoroughly with warm water and soap. Treatment with imiquimod cream was continued until the visible lesions disappeared, but not more than 16 weeks.

Vulvectomy was performed as follows: patients were placed in the obstetric position; an incision was made 3 cm above the pubis, covering the labia to the posterior fascia, and the vulvar tissue with underlying tissue and lesions were separated; the drug was removed; a series of sutures and an aseptic dressing were applied to the skin.

Some women underwent surgical excision of the affected part of the vulva, taking into account that the disease usually spreads more widely than what is visible on the skin or vulvar mucosa and leaving a margin of 2 cm. After excision of the affected area, 6 sessions of PDT with 5-aminolevulinic acid every 2 weeks or treatment of the vulva with 5% imiquimod ointment (as described above) were sometimes additionally performed.

The effectiveness of treatment was assessed basing on the presence/absence of complaints, visual assessment of changes in the area of treated pathological lesions and morphological examination at 3, 6 months and 1 year after treatment:

- complete regression (CR) - the absence of all signs of the disease after 100% regression of pathological lesions 3 months after the treatment, confirmed histologically 6 months after the treatment and 1 year;

- partial regression (PR) - reduction in the total size of pathological lesions by 50% or more with subsequent stabilisation, established in 3 months and confirmed histologically in 6 months and 1 year;

- no effect (NE) - a decrease in the total size of pathological lesions by less than 50%, a condition without a decrease or increase in the area of pathological lesions.

Results and their discussion

The classification of women by type of treatment depending on the diagnosis is presented in Table 1. Out of 262 patients, the radical approach (vulvectomy) was used in only 10 women or 3.82% of the total number. It should be noted that all of these women were diagnosed with intraepithelial neoplasia of the vulva, which is independent of HPV. Surgical excision of the pathological lesion was performed in 7 (2.67%) women, and in most cases (71%) with dVIN.

This method was used for only 1 patient with VHSIL (14%) and 1 (14%) with Paget's disease of the vulva. A combined approach to treatment was used for 11 patients, namely, for 4 (1.53%) excision with 5% imiquimod cream, and for 7 (2.67%) excision with PDT with 5-aminolevulinic acid. Moreover, excision with imiquimod treatment was used only for VHSIL (in 2 or 50% of cases) and for Paget's disease of the vulva (in 2 or 50% of cases), while excision with PDT with 5-aminolevulinic acid was used mainly for dVIN (in 5 of 7 cases or 71%). Thus, different types of surgical intervention were used in 28 (10.69%) women with vulvar neoplasia, most of whom (20 patients or 71.43%) were diagnosed with dVIN.

Almost two-thirds of patients in our study received PDT with chlorine E6 (n=179 or 68.32%), with 67% (120 women) having dVIN and 31% (55 women) having Paget's disease of the vulva. Imiquimod 5% cream was used as a treatment by 32 patients, the vast majority of whom (30 women or 94%) had vulvar HSIL.

Thus, in our study, patients with dVIN got the following treatments: excision of the pathological lesion; excision followed by PDT with 5-aminolevulinic acid; vulvectomy and PDT with chlorine E6. Patients with VHSIL had excision; excision with PDT with 5-aminolevulinic acid; excision with 5% imiquimod cream; PDT with chlorine E6; treatment of

the pathological focus with 5% imiquimod cream. Excision, PDT with chlorine E6, imiquimod cream, and excision followed by imiquimod cream were used to treat grade Ia vulvar Paget's disease.

Table 1 - Classification of patients by therapy type depending on diagnosis (n, %)

Method of treatment	Number of patients treated						
	Total, n	including with the following diagnoses					
		dVIN		Vulvar HSIL		Paget's disease	
		n	%	n	%	n	%
Excision	7	5	71	1	14	1	14
PDT with chlorine E6	179	120	67	55	31	4	2
PDT with 5 aminolevulinic acid	23	15	65	8	35	0	0
Imiquimod 5% (injectable)	32	0	0	30	94	2	6
Combined (excision + imiquimod 5%)	4	0	0	2	50	2	50
Combined (excision + PDT with 5-aminolevulinic acid)	7	5	71	2	29	0	0
Vulvectomy (partial / complete)	10	10	100	0	0	0	0
Total	262	155		98		9	

In all cases with intravenous photosensitizer, no symptoms of skin phototoxicity (itching, hyperaemia of exposed areas of the body, swelling of the soft tissues of the face, etc.) were noted. During the PS infusion and the period before the PDT session, the general condition of the patients was satisfactory. There were no allergic reactions accompanied by severe dysfunctions of vital organs (Heinrich Quincke's edema, urticaria, drop in blood pressure, bronchospasm). Despite the premedication prior to the PDT session, the patients had moderate pain (I-II degree). In the post-procedural period, all patients were prescribed analgesics (ketorolac tromethamine 30 mg, dexketoprofen tromethamol 73.8 mg).

After the end of the PDT session, all patients had moderate swelling in the areas of pathological tissues exposed to photoirradiation. Within 1-5 days after the treatment, the formation of a dark brown or black photochemical necrosis zone was observed.

During photodynamic therapy with topical application of PS in the form of 6% 5-aminolevulinic acid phosphate gel, patients individually perceived different levels of pain during treatment: from mild burning or stinging to unbearable pain. After the sessions for 2 days, the patients noted hyperaemia and slight swelling in the vulvar area with mild burning pain.

The effectiveness of the therapy was assessed clinically and morphologically in 3, 6 and 12 months. The results of the follow-up of patients with dVIN after partial or complete vulvectomy in 3 and 6 months in all cases were assessed as complete regression clinically and morphologically. A year later, only 1 woman (10 %) had recurrence of complaints and a morphological recurrence of the disease.

When using a less radical approach to surgical treatment, namely, excision of only the pathological lesion, 6 (85.71%) patients had complete regression, and 1 (14.29%) had partial regression with morphologically confirmed recurrence. In the course of follow-up, the proportion of women with recurrence increased to 28.57% (n=2) in 6 months and 42.86% (n=3) in a year. In other words, only 57.14% of patients with excision of the pathological lesion after 12 months showed complete regression of the disease.

According to the latest European consensus on preinvasive vulvar lesions (2022), due to the risk of rapid progression of dVIN to invasive squamous cell carcinoma of the vulva [2, 9], medical treatment or ablation of dVIN is not recommended and conservative excision with negative surgical margins followed by ongoing surveillance should be preferred [10, 11]. At the same time, the effectiveness of this approach varies between authors for many reasons, including differences in patient-related factors (multifocal disease, immunosuppression, and smoking) and the type of VIN. In addition, methodological limitations and differences in statistical analysis between studies contribute to the significant variability of the data. Other authors have reported high rates of recurrence after surgical excision. For example, Satmary W. et al. recorded up to 50% of recurrences within 16.9 months, which, in their opinion, requires more careful monitoring of patients during the first 2 years after surgery, especially those over the age of 50 [12].

Therefore, drug therapy is a therapeutic option that is suitable to preserve normal vulvar anatomy and avoid disability [9]. Imiquimod cream with 5% was used in our study mainly in women with VHSIL and showed a fairly high efficacy during treatment. While only partial regression was recorded in almost all cases (93.75%, n=30) in 3 months, complete regression occurred in 71.87% (n=23) in six months and in 81.25% (n=26) in a year. It should be noted that morphologically, in 3 months, the disease recurrence was recorded in only one third of patients (31.25%) with clinically assessed partial regression (93.75%), while over time, a complete coincidence of these indicators was recorded. This may be due to the fact that topical treatment with imiquimod cream requires a rather long therapy and changes in tissues occur gradually.

Imiquimod is an immune response modifier that targets TLR-7, which stimulates the secretion of pro-inflammatory cytokines by dendritic cells, thereby causing a strong immune infiltration [13]. After an 87% complete or partial response rate in patients participating in a pilot study [14], two randomised controlled trials comparing imiquimod with placebo were conducted [15, 16]. The complete response rate in women receiving imiquimod was 81% in the study by Mathiesen O. et al. [15] and 35% in the study by Van Seters et al. [16] within 2 to 5 months after treatment. Only van Seters et al. reported follow-up data in 12 months: 35% of patients with a complete response (n = 9) in the imiquimod group compared to 0% in the placebo group; no difference in the rate of progression to invasive disease between the two groups (1/26 vs. 2/26) [16]. Long-term follow-up of the initial group from the study by van Seters et al. was available [17]: eight of the nine initial patients with a complete response did not relapse after a median follow-up of 7.2 years. The efficacy of topical imiquimod 5% cream as a conservative treatment for Paget's disease of the vulva with minimal side effects has also been demonstrated. Researchers reported a complete response ranging from 22% to 90% of cases [18, 19, 20]. Treatment protocols vary from study to study (1-5 times per week, from a minimum of 3 weeks to a full year), but the total treatment duration was 16 weeks [18, 19].

Combined therapy with imiquimod in our study showed high efficacy both clinically and morphologically with complete regression of the disease (100%) and no recurrence during the year of follow-up in all patients treated with this approach. Whereas, according to some other authors, the combination of cold knife surgery and imiquimod cream as an adjuvant does not provide benefits in terms of reducing the recurrence rate [21], but may allow for a smaller excision area for better preservation of anatomy and function.

According to our data, the clinical evaluation of photodynamic therapy with 5-aminolevulinic acid showed no effect after 3 months of treatment in any of the cases (n=23). Morphologically, the majority of them (18 women or 78.26%) had a recurrence of the disease in 3 months. On the other hand, combined therapy with PDT and 5-aminolevulinic acid with the presence of complete regression and no relapse in 3 and 6 months showed an efficacy decrease in the dynamics. In a year, almost half of the patients (57.14%) had partial regression and morphological relapse.

Different PDT trials for the treatment of VIN are characterised by non-standardised methodology regarding the type of photosensitizer, the way of administration, duration of PD application, type and wavelength of light source, and number of treatment cycles per patient. In addition, and most importantly, different definitions of treatment response are used.

According to various authors, the overall clinical response to topical PDT ranges from 31.2% to 56% [22, 23, 24]. The relapse rate ranges from 14.3% [24] after an average of 13 months to 48% [25] after an average of 53.7 months of follow-up. Only one study reported a 9.4% post-treatment invasion rate [22].

A significant number of patients in our study (n=179 or 68.32%) were treated with systemic PDT with chlorine E6. This type of therapy showed a complete regression of the pathological focus clinically and morphologically in 96.65% (n=173) of women in 3 months.The same indicators were maintained after 6 months. While in a year, the percentage of women with partial regression with morphologically confirmed recurrence increased slightly to 15.08%. However, a greater proportion of patients (84.92%) had complete regression and no recurrence of the disease (Figures 1, 2, 3).

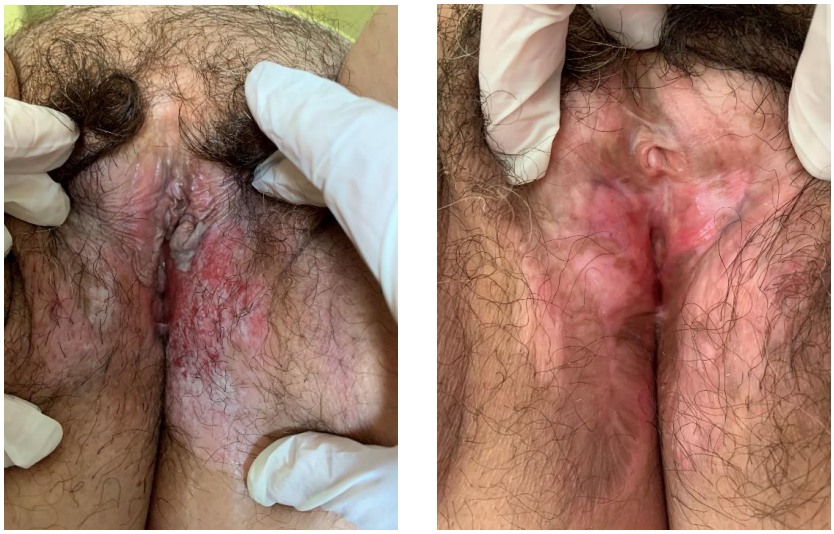

Fig.1. Paget's disease of the vulva before and after PDT with chlorine E6 in 1 year

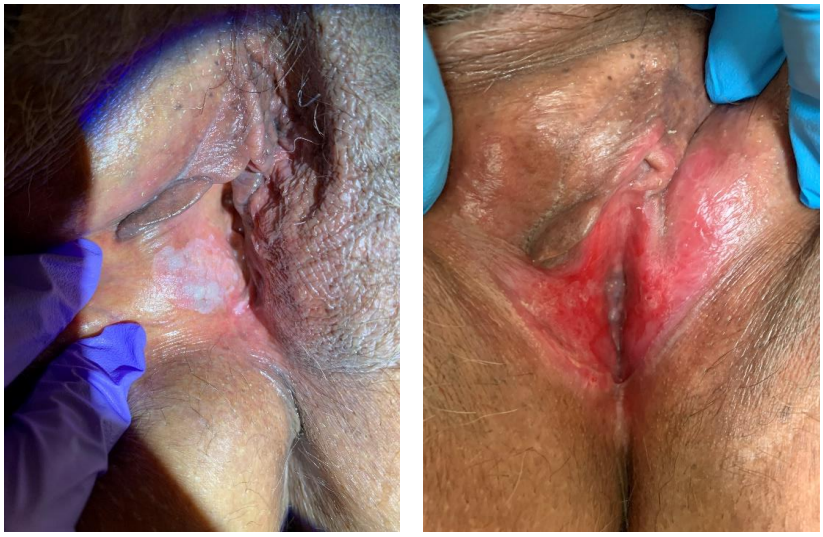

Fig.2. HSIL of the vulva before and after PDT with chlorine E6 in 3 months.

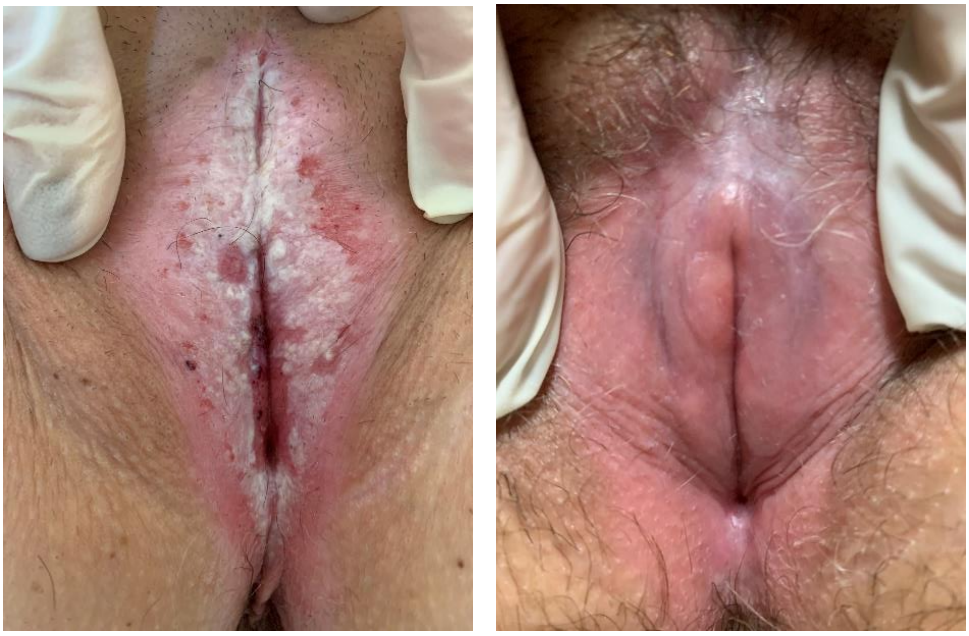

Fig.3. Vulvar dVIN before and 6 months after PDT with chlorine E6

There are few studies on the systemic use of PS. Another study evaluated the effects of the systemic photosensitising agent meta-tetrahydroxyphenyl chlorine (mTHPC) in high-grade VIN. Six patients received an intravenous dose of 0.1 mg/kg body weight of mTHPC, and the VIN area was irradiated 96 hours later with 652 nm light from a diode laser. Six months later, two patients had a recurrence of VIN in the same area, and one patient got a new VIN lesion. They were treated with either further PDT or excision. Two years later, no recurrence of VIN in the same area was reported in all patients who were followed up. In other words, in this study,using PDT with mTHPC demonstrated good cosmetic and functional results, showing a great advantage over surgery [26].

Conclusions. Modern methods of conservative treatment of vulvar intraepithelial neoplasia using imiquimod cream and systemic photodynamic therapy can be a highly effective alternative to surgery.

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Informed Consent Statement: Written informed consent for treatment, use of the patients' personal data and their use was obtained from all examined women.

Data Availability Statement: All information is publicly available, data on a specific patient can be obtained upon request from the author.

Conflict of Interest: The author no conflict of interest.

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