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Original Research

Medical treatment as a possible treating modality for premalignant vulvar lesions

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Summary

Purpose of investigation: Purpose of this investigation was to determine the possibilities of non-surgical, medical treatment of high-grade squamous intraepithelial lesion (HSIL) of the vulva and differentiated type of vulvar intraepithelial lesions (VIN) and to determine the effect of medical treatment by local and systemic immunomodulators in relation to different histological types. *Materials and Methods:* The study included five patients with different histological types of VIN. The patients were given: systemic immunomodulator and antivirotics. After that, the patients initially included in the study were followed for a year, while the follow-up of the older patients group is still ongoing, and it has been six months since the completion of the treatment. *Results:* Combined medical therapy has positive therapeutic effect in the treatment of young HPV-positive adolescent patients with HSIL of the vulva. Medical treatment in HPV negative women with differentiated type VIN is less efficient, achieving partial remission in only 33% of patients. *Conclusion:* Combined medical therapy achieved better effects in hyperpigmented multifocal type of VIN.

Key words: Medicals treatment; Vulvar intraepithelial neoplasia; Adolescence; Menopause; Premalignant lesions.

Introduction

Vulvar intraepithelial neoplasia (VIN) is a premalignant disease of the squamous epithelium, showing a proliferation of abnormal keratinocytes of the vulvar epidermis without invasion of the basement membrane. Abnormal keratinocytes have hyperchromatic nuclei, irregular nuclear membranes, disturbed nucleoprotoplasmatic relation, and often showing the cytopathogenic effects of HPV infection. Depending on the thickness of the epithelium affected by these atypical keratinocytes, analog to cervical ones, VIN is considered as a separate entity.

Primary division of VINs was analogous to the division of cervical intraepithelial lesions (CINs) on: VIN I, VIN II, and VIN III. Considering the fact that VIN I represents cytopathogenic effects of HPV infection only, in 2004 International Society for the Study of Vulvovaginal Disease (ISSVD) reclassified VIN into a single-grade system, including only high-grade intraepithelial lesions of the vulva (VIN II and VIN III) [1].

Histologically, VIN appears in two different etiopathogenic types. The first one is the usual-type VIN that is more common in younger women and is related to high-risk, oncogenic HPV types, smoking, and immunodeficiency. Usual-type VIN is subdivided into warty, basaloid, and mixed types. The second one is a differentiated type VIN that tends to occur in older, postmenopausal women, associated with chronic vulvar dermatoses, lichen sclerosus, and is most commonly HPV-negative. Better prognosis and higher rates of spontaneous regression are reported in vulvar neoplasia of the first usual-type VIN. The second, differentiated type VIN is more commonly associated with invasive carcinoma and high recurrence rates are common [2, 3].

In 2015, ISSVD changed the terminology and introduced the term vulvar squamous intraepithelial lesions. The usual type of VIN are classified as high-grade squamous intraepithelial lesion (HSIL) of the vulva, while flat condyloma, basal atypia and koilocytic changes are classified as low-grade squamous intraepithelial lesion (LSIL) of the vulva. Differentiated type of VIN remains the same [4, 5].

Since vulvar intraepithelial neoplasia is most commonly asymptomatic, or presents with non-specific clinical manifestations (itching, burning, dyspareunia), the exact incidence of the disease is unknown, and literature data are considerably contrasting. Still, the development of colposcopy and histological evaluation of clinically suspected recurrent vulvar condylomas resulted in a conclusion that the incidence is increasing, especially in women younger than 50 years of age. Some large epidemiological studies reported a four-fold increased incidence of VIN between

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1973 and 2000 [6]. Besides the rise in the incidence of HPV infection, the reason for the increase in incidence of patients with VIN is also the lack of clear recommendations for the detection and screening, as well as the diversity of clinical manifestations of VIN.

The etiopathogenesis of VIN is multifactorial and depends on histological type. Nevertheless, the underlying etiological factor is reported to be HPV infection with highly oncogenic viruses, as well as chronic inflammation (Lichen) and cigarette smoking. Just for these etioepidemiological reasons, HPV vaccination, treatment of chronic vulvar dermatoses and reduction of smoking are recommended as measures of primary prevention.

The aim of the paper was to: 1) determine the possibilities of nonsurgical, medicamentous treatment of HSIL of the vulva and differentiated type VIN and 2) determine the effect of medicamentous treatment of VIN by local and systemic immunomodulators in relation to different histological types.

Materials and Methods

This paper presents initial experiences of a planned large study on possibilities of medical treatment of VIN. Literature data reveal mostly local antivirus drugs and immunological modulators as possible modalities of VIN medical treatment. In the present study the authors aimed to examine common therapeutic effects of local and systemic immunomodulators. Due to different histological types of VIN, different etiopathogenesis and clinical presentations, the study included patients of different age, clinical form, and HPV status.

This study was conducted in Clinic for Gynecology and Obstetrics, Clinical center Nis, Serbia. VIN is an extremely rare disease in young women (especially in adolescence). The reason why the present authors conducted this study was just because the first patients were in this age group. The first one who was referred for an examination was 16-years-old with HSIL of the vulva, and the other one was 18-years-old, also with usual type, but HSIL of the vulva. They were both HPV-positive to highly oncogenic HPV viruses, had verrucous type of VIN, and multifocal hyperpigmented clinical forms.

Since the present authors aimed to examine the effects of medical treatment to other histological types of VIN in older patients as well, three more menopausal patients were included in the study. One of them had a differentiated type VIN of multifocal form, the second one had a differentiated type VIN of unifocal localization, and the third one had a unifocal VIN, but involving greater area (most part of the right unilateral labia pudenda). Neither of these three patients had hyperpigmented form of the disease and they were HPV negative.

Before the treatment, all the patients underwent colposcopy examination and biopsy that excluded the presence of invasive disease, and HPV tests were performed by the PCR method. The parents of the adolescent girls were informed on possible treatment risks, current protocols on VIN treatment, and planned medical treatment and follow-up.

The patients were given combined medical therapy. Systemic immunomodulator and antivirotics, Inosine acedoben dimepranol 50 tablets, 500 mg, 3×2 tablets daily, were given to the patients orally, and Imiquimod 5% cream was locally applied with a cotton swab to the lesions, including healthy skin within 1 cm of the lesion, once daily at bedtime, three times a week (every other day) for up to 12 weeks, with regular vulvoscopy check-ups every four weeks. After that, the patients initially included in the study (younger adolescent patients) were followed for a year, while the follow-up of the older patients group is still ongoing, it has been six months since the completion of the treatment.

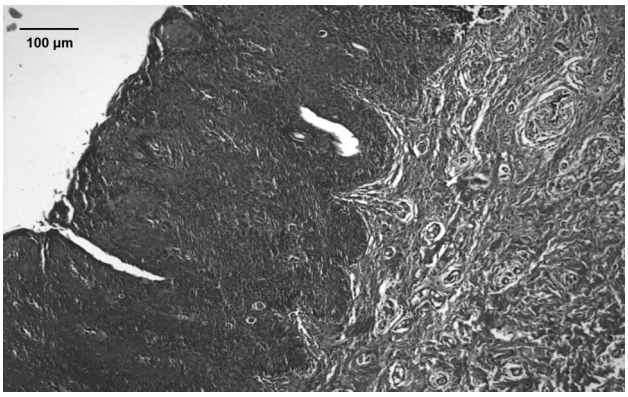

Figure 1. — Histopathological picture of usual-type of vulvar intraepithelial neoplasia III (verrucous form) (×200).

This investigation and treatment protocol have been approved by the Hospital Ethics Committee of Clinical center Nis. Written informed consent was also obtained from all participants.

Results

The first patient with medically-treated VIN was 16-years-old, and in her case vulvar condylomas were repeatedly treated with laser vaporization. Vulvoscopic examination revealed a suspicious atypical hyperpigmented multifocal change that required histopathological evaluation. Targeted biopsy was performed and the diagnosis was HSIL of vulva, as seen on Figure 1, and HPV test was positive for HPV type 16. The patient was treated with Imiquimod and Inosine acedoben dimepranol according to previously described scheme and had regular vulvoscopy check-ups every four weeks for 12 weeks. After 12 weeks, complete remission of the disease was achieved**.** A narrow banding hyperpigmented field left after the treatment was excised and sent for HP examination. Histopathological finding (Figure 2) showed benign changes only. HPV test was negative six months post-treatment.

The second young patient also had complete regression of the HSIL of the vulva after completion of medical treatment. Among the menopausal women, there were two patients with multifocal differentiated type VIN and one with differentiated type VIN changes and they did not respond to treatment, while the third patient with differentiated type VIN unifocal change had partial remission of the disease.

Complete remission of the disease (CR) was registered in two patients (40%), partial remission (PR) in one (20%), and no response (NR) was detected in two patients (40%). Multifocal differentiated VIN form was present in three pa-

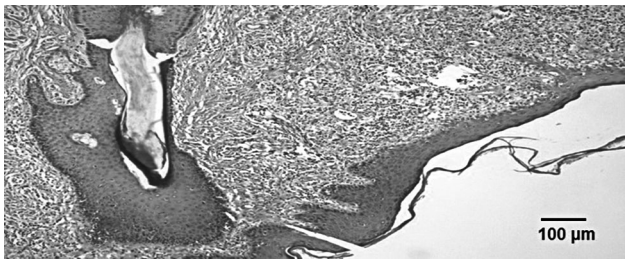

Figure 2. — Histopathological finding after 12 weeks (benign changes only, Lichen type) (×100).

tients (60%), while two patients (40%) had a unifocal change. In 66% of multifocal changes (two out of three) there was CR, while unifocal changes showed PR in 50% of patients, and in 50% there was no therapeutic response. Hyperpigmented clinical manifestation of VIN was registered in two patients (40%). They were both young adolescents with HSIL of the vulva and they both had a 100% therapeutic response. Only 33% of patients with non-pigmented form of the disease had PR.

Discussion

Summing up, the obtained results showed that the best therapeutic response to medical treatment of vulvar neoplasia was registered in younger, HPV-positive patients with multifocal, hyperpigmented, type of VIN. Good therapeutic response of hyperpigmented lesions to non-surgical treatment was described in a large Korean study, where hyperpigmented lesions were evident in 55.6% of patients and all of them achieved CR [7]. In one-year follow-up, no recurrences were detected. Further vulvoscopic check-ups have been planned.

Both diagnostic and therapeutic approaches regarding VIN lack clear recommendations, consensus, or criteria. Nevertheless, in order to avoid an occult invasive process, surgical treatment has been proposed as the standard treatment of VIN (local excision, vulvectomy) to ensure excision margins and to exclude invasive disease. Thus, in about 3% of patients who underwent surgery for VIN, occult invasive cancer was detected [8].

Surgical treatment signifies excision with up to 1 cm of healthy tissue. Vulvectomy is recommended only in confluent multiple lesions and in immunocompromised patients. The status of obtained excision margins into the healthy tissue is an important prognostic and therapeutic factor responsible for the results of the treatment. The percentage of positive margins after surgical excision of VIN described in literature is high, from 24% to 68%. The reason for such high rates are due to non-specific clinical manifestations of VIN, undefined margins of the pathological process on the skin of the vulva, and subclinical manifestations of HPV infection in surrounding tissue. Inadequate treatment results and the disease recurrences are more common in the group of patients with positive excision margins, with rates over 50% [9, 10].

Although officially recommended, surgical treatment is not ideal. Its consequences include physical, psychological, social, and sexual traumas. On the other hand, high rate of positive surgical margins on excision and high recurrence rates indicate the need for other non-surgical treatment modalities as well [11, 12].

Non-surgical treatment modality is reserved for cases where invasive disease is excluded by vulvoscopic examination and biopsy. Lesions with atypical blood vessels, or showing rapid change in colour from red to brown and black, and those that rapidly change borders or size are suspicious for progressive intraepithelial change that requires multiple biopsies and excisions. The presence of condylomatous changes in postmenopausal women, as well as the presence of condylomas unresponsive to classical topical therapy at any age, also require biopsy and histological evaluation.

Considering the most common etiopathogenesis of VIN to be of viral origin, non-surgical options include application of antiviral drugs and treatments: imiquimod, cidofovir, indole-3-carbinol, 5-fluorouracil, interferon-α/γ, podophyllotoxin, inosine acedoben dimepranol, photodynamictherapy and laser ablation. The advantage of this treatment modality is in its effect on surrounding normal tissue, resulting in an increase of local immunological factors, thus, unlike surgical treatment, significantly reducing the recurrence rate [13].

The fact is that VIN has a slow rate of progression, but it is also a fact that available detection methods by visual inspection, vulvoscopy, toluidine blue, and cytology cannot predict with certainty the nature of each VIN lesion. Nevertheless, according to well known facts on etiopathogenesis and the nature of the disease, it can be concluded that expectative and conservative approaches are encouraged, along with proper follow-up and previous exclusion of suspicion of invasive disease. Considering the fact that HPV infection is one of the most common etiological factors for VIN (since it acts through both mutagenic and immunosuppressive effects, reducing the number and efficacy of immunopresenting dendritic cells, natural killer cells, and T-cells), further progression or recovery is associated with HPV negativization [10]. Negativization of HPV infection in patients treated for VIN can be confirmed by HPV test, or by histologic reduction of p16INK4a expression [14].

VIN may spontaneously and without treatment regress to a certain degree (about 16%). The highest rate of spontaneous regression is seen during pregnancy and in the postpartum period. Just for these reasons, it is necessary to employ a more expectative approach for this group of patients [15].

One of the non-surgical treatment modality employs laser ablation. Laser ablation, as well as other forms of non-sur-

gical treatment, is only appropriate if the presence of suspicious invasive disease has been excluded. Recurrences are more common than in excision. A special problem for laser ablation is the treatment of areas with hair follicles which can contain skip lesions, so at least a 3-mm depth through the dermis is required in these areas for laser ablation treatment. For these reasons the utilization of laser in hair-bearing and large areas is limited [16].

Medical therapy described in literature as a non-surgical modality in the treatment of VIN most commonly shows the application of Imiquimod and Cidofovir. Positive therapeutic effects of other medications used in negativization of HPV infection have been described (indole-3-carbinol, 5-fluorouracil, interferon-α/γ, and podophyllotoxin).

The first medication the authors used in this study with proved systemic effects, as well as an effect in eradicating HPV infection, and as an agent showing immunomodulatory activities, is Inosine acedoben dimepranol. Its effect is based on immunomodulation since it binds to purinergic receptors in immune cells and initiates production of cytokines which activate antigen-presenting cells, and T-lymphocyte differentiation to Th1 immunological response. The drug also has antiviral effects since it inhibits viral RNA, reduces viral mRNA (by replacing guanine in adenosine with inosine) and enzyme (digidroproteoatsynthetase) involved in viral replication, inhibits the attachment of polyadenylic acid to viral m RNA, and increases translation of proteins to lymphocytic mRNA. Although there are no available papers in literature so far, due to its antiviral and immunological effects, the drug may have effect the treatment of VIN in combination with local immunomodulator Imiquimod.

The second medication the present authors used that can also have effects on local eradication of HPV infection is Imiquimod. It is heterocyclic imidazoquinoline amide with a role to modify immune responses by activating toll-like receptors (TLR)-7 and TLR-8 cascade. By having an impact on surface receptors of immature plasmacytoid dendritic cells, it results in their maturation and activation. Activated dendritic cells induce production of cytokine mediators which activate T-cell immune response believed to be directly responsible for HPV elimination. Furthermore, this drug has a direct pro-apoptotic effect on tumour cells and antiviral activity against HPV [17].

Positive therapeutic effect of Imiquimod is demonstrated as a concentration increase of immunopresenting cellular immunity cells: CD1a+ dendritic cells, CD8+ T cells, and CD94+ natural killer cells, not only in the VIN zone, but also in surrounding healthy tissue where skip lesions may be present. Such a broad immunological reaction and immunostimulatory effect that is continued after the application of the drug is certainly one of the reasons for lower recurrence rates after the application of Imiquimod in comparison to surgical treatment. Thus, the rate of VIN recurrences after the treatment with Imiquimod cream is lower than the rate of recurrences after surgical excision (15% *vs.* 42%) [18]. In addition to immunological and antiviral effects and evident reduction in VIN lesions size, Imiquimod relieves the pain and pricking sensation associated with VIN; unlike surgical treatments, it results in better cosmetic appearance, thus creating a considerably lower psychosexual dysfunction [10]. Due to such therapeutic effects, Imiquimod 5 as an immune response-modifying agent was approved by the Food and Drug Administration (FDA) for the treatment of actinic keratosis, superficial basal cell carcinoma, and external genital warts. Official guidelines, recommendations and protocols on Imiquimod application for the treatment of VIN have not yet been determined.

Therapeutic effect of Imiquimod depends on histological type, size, multifocality, and lesion hyperpigmentation. Hyperpigmented form occurs in 10% to 15% of VINs (more common in Asian women). This form shows the best therapeutic response to Imiquimod. In non-pigmented lesions CR is 25%, whereas in patients with pigmentation, CR is over 60%. In the present study there were 40% hyperpigmented VIN lesions, with achieved therapeutic response of 100%.

As for the enlargement of the change, literature data show that unifocal lesions less than 3 cm in diameter have better therapeutic responses (66.7: 34%) in comparison to enlarged multiple lesions. In the present study better therapeutic response was achieved in multifocal than in unifocal lesions (66% *vs.* 50%), but it should be mentioned that one patient with differentiated type VIN had unifocal change more than 3 cm in diameter.

Smoking and age (65 and older) associated with previously described unfavourable clinical manifestations of the disease (enlargement, multifocality) may also have unfavourable therapeutic outcome and higher recurrence rate [19].

Side effects of the use of topical Imiquimod, including pruritus and burning, occur in about 33.3% of patients. Literature data show that just these patients with severe reactions and more expressed side effects achieved better therapeutic response to Imiquimod and lower recurrence rates [20].

Generally speaking, all the patients treated for VIN, regardless of treatment modality, are at increased risk of recurrences, but given the relatively slow rate of the disease progression, it is still possible to follow-up the patients every three months initially, then six-monthly, and yearly thereafter. Most studies and available literature do not provide the exact rate of recurrences, since the follow-up is mostly limited to the period of 36 months at most.

Conclusion

In conclusion combined medical therapy with local and systemic immunomodulators (Imiquimod and Inosine acedoben dimepranol) have a positive therapeutic effect in the

treatment of young HPV positive adolescent patients with HSIL of vulva. Medical treatment with local and systemic immunomodulators in older HPV negative menopausal women with differentiated type VIN is less efficient, with achieved partial remission in only 33% of patients. Combined medical therapy with local and systemic immunomodulators achieved better effects in hyperpigmented multifocal type of vulvar intraepithelial neoplasia. It is necessary to conduct a multicentric, heterogeneous study enrolling a greater number of patients of different age, histological type, grade, and HPV status as well, in order to reach a joint consensus and treatment protocol on the use of combined medical treatment with local and systemic immunomodulators in the treatment of VIN.

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