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Adjuvant topical treatment with imiquimod 5% after excisional surgery for VIN 2/3

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Abstract. – OBJECTIVE: Vulvar intraepithelial neoplasia (VIN) is a premalignant lesion of the vulva. The incidence of VIN is increasing. The surgery is currently the gold standard therapy for VIN, but Imiquimod could be a completion to surgery. The aim of this study is to compare the overall complete response, the recurrence rate and the risk factors for recurrence among two groups of patients: women with high grade VIN underwent surgery and patients treated with surgery plus Imiquimod.

PATIENTS AND METHODS: 80 patients with histologically diagnosed VIN 2/3 were enrolled in this prospective study. Our patients were divided into two groups: 40 women underwent surgery (A) and 40 patients were treated with surgery plus Imiquimod (B). All women had a 5-year follow-up. Recurrence rate and complete response were evaluated. The following patients' characteristics were analyzed: smoke, multifocal disease, multicentric disease, degree of the lesion.

RESULTS: In the group A recurrence rate was 44.8%, in the group B it was 48.4%. In both groups the presence of multifocal lesions (p = 0.02) and VIN 3 (p = 0.006) before treatment was associated with a higher risk of recurrence.

CONCLUSIONS: This study found that surgery remains the principal approach for VIN with regard to relapse and complete response since the treatment with Imiquimod associated with surgery didn't show a lower recurrence rate. Although the surgical treatments remain the best therapeutic option for VIN with regard to recurrence and overall complete response, the combined therapy seems to be an interesting modality, but further studies are needed.

Key Words:
VIN, Imiquimod, Cold knife excision, Surgery, Recurrence, Response.

Introduction

Vulvar intraepithelial neoplasia (VIN) is a premalignant lesion involving the vulva. It consists of the presence of abnormal keratinocythes in the vulva that have the potential to develop into invasive carcinoma. The incidence of VIN is increasing, with 60-75% occurring in young women. The risk of progression from VIN to invasive cancer is 3 to 9%[1]. The classification of VIN 1 (mild dysplasia), VIN 2 (moderate dysplasia), and VIN 3 (severe dysplasia) is no longer used. In 2004 the International Society for the Study of Vulvovaginal Diseases (ISSVD) reclassified VIN into two groups: usual type and differentiated type[2]. The differentiated type is tipically lichen sclerosus related and affects older women. The usual type is the most frequent variant and it is caused by high-risk HPV, usually appears with multifocal lesions and occurs in younger women. The histological characteristics are poorly to undifferentiated basal cells and highly atypical squamous epithelial cells involving the entire thickness of the epithelium with or without a warty and hyperkeratotic surface.

The surgical treatment of all visible lesions is the standard therapy for VIN and includes several techniques: laser ablation or excision, knife local excision, simple vulvectomy and skinning vulvectomy[3]. However, surgical treatment often leads to disfigurement of female external genitalia and impaired sexual function[4]. In addition, recurrences are common after surgery, since the underlying cause, a persistent HR HPV infection, is not cleared[5]. Imiquimod was recently introduced as an alternative for surgery[6]. It is an im-

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mune-response modifying drug that induces innate and cell-mediate immunity, thus eliciting antiviral and antitumor activity. It is safe and effective in the treatment of external genital warts caused by HPV. Several studies have evaluated the response and recurrence of VIN in patients treated with Imiquimod[7-9].

The aim of this study is to compare the overall complete response, the recurrence rate and the risk factors for recurrence among patients with high grade VIN surgically treated (cold knife excision) and patients treated with surgery plus Imiquimod. It is also the pursuance of a previous study, which compared surgery versus Imiquimod for the treatment of VIN 2/3 usual type, finding the superiority of surgery[10].

Patients and Methods

Since 2000 to 2012, from all the Universities and Country Hospitals participating in the study, a total of 80 patients with histologically diagnosed usual type VIN2 and VIN3 were enrolled in this prospective study. All the women gave their informed written consent. The histological diagnosis of VIN usual type was made according to the ISSVD. Patients with recurrent VIN, women treated more than once, immunocompromised patients, differentiated type VIN lesions and VIN1 lesions were excluded from the study. Patients were divided into two groups: 40 women underwent surgery (group A) and 40 patients were treated with surgery and then Imiquimod (group B). Surgery was performed by cold knife excision trying to tailor the excision to the lesion as much as possible. However at least 5 mm free-margin was guaranteed during the procedure[11]. All the margins of lesions were free from disease. Imiquimod 5% (250 mg) was applied locally by the patient one month after surgery twice a week for 16 weeks. All women were seen every 6 months for a 5-year follow-up. Recurrence rate and complete response were evaluated. We have considered as recurrence the onset of a lesion after an initial complete response to the treatment. The following patients' characteristics were analyzed, in order to evaluate if one of these would increase the risk of recurrence: smoke, multifocal disease (more lesions in the vulva), multicentric disease (lesions at many sites of the lower female genital tract), degree of the primary lesion (VIN3). The above mentioned variables were also compared between the two groups of patients.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows, version 10 (SPSS Inc., Chicago, IL, USA). As the sample size was not enough to perform a multivariate analysis, only univariate analysis was done. Variables were analyzed using the Student's *t* test and the Chi Square Test. The risk was assessed using the Relative Risk (RR), with Confidence Intervals (CI) 95%. $p < 0.05$ was considered statistically significant.

Results

In the group A 2 patients were lost during follow-up, while in the group B 3 women were lost during follow up and 4 were ruled out from the study for the side effects of Imiquimod. So 38 patients treated with surgery and 33 patients treated with surgery plus Imiquimod were analyzed. The mean age of group A was 40.1 years. Smokers were 52.7% (20/38) of the patients. The degree of the initial lesion was VIN3 in 60.6% (23/38) of cases. Multifocal lesions were 71.1% (27/38). Multicentric lesions were observed in 42.1% (16/38) of these patients. Recurrence rate was 44.8% (17/38). Mean time of recurrence was 29 ± 26 months. Overall complete response was 55.2% (21/38). The mean age of group B was 41.3 years. Smokers were 45.4% (15/33). The degree of the initial lesion was VIN 3 in 63.6% (21/33) of cases. Multifocal lesions were 69.7% (23/33). Multicentric lesions were observed in 39.4% (13/33) of women. Recurrence rate was 48.4% (16/33). Mean time of recurrence was 31 ± 30 months. Overall complete response was 51.6% (17/33). In both groups, the presence of multifocal lesions ($p = 0.02$) and VIN 3 ($p = 0.006$) before treatment was associated with a higher risk of recurrence, while smoke ($p = 0.4$) and multicentric lesions ($p = 0.22$) did not increase the risk. The recurrence rate was similar in both groups ($p = 0.7$). The main results are summarized in Table I.

Discussion

The treatment of VIN is directed at relieving symptoms, preserving normal anatomy and function, and preventing the development of invasive disease. Although surgery is the standard therapy for patients with VIN, new medical therapies are being investigated. These are mostly 5-

Table I. Treatment outcomes and risk factors for recurrence.

	Surgery n = 38	Surgery + Imiquimod n = 33	p value
Recurrence	17/38 (44.8%)	16/33 (48.4%)	0.7
Complete response	21/38 (55.2%)	17/33 (51.6%)	0.7
Disease free	29 ± 26	31 ± 30	0.7
Age	40.1 ± 2.6	41.3 ± 3.6	0.1
Smoke	20/38 (52.7%)	15/33 (45.4%)	0.54
Multifocality	27/38 (71.1%)	23/33 (69.7%)	0.9
Multicentric lesions	16/38 (42.1%)	13/33 (39.4%)	0.81
VIN 3	23/38 (60.6%)	21/33 (63.6%)	0.78
Risk factors	**Patients n = 71**	**RR (95% CI)**	**p value**
Smoke	35/71 (49.3%)	1.23 (0.75-2.04)	0.4
Multifocality	50/71 (70.4%)	2.27 (1.01-5.08)	0.02
Multicentric lesions	29/71 (40.8%)	1.36 (0.83-2.23)	0.22
VIN 3	44/71 (61.9%)	2.28 (1.15-4.51)	0.006

fluorouracil, cidofovir and the recent self-applied immunotherapy with 5% Imiquimod cream[12]. HPV infects epithelial keratinocites and the virus does not elicit cell death, making it difficult for the host immune system to recognize the virus during early stages of infection[13,14]. Imiquimod modulates the immune response by binding on Toll-like receptors on the surface of dendritic cells, thereby, inducing secretion of pro-inflammatory cytokines favouring the clearance of a persistent HPV infection[15,16]. Several studies have indicated that Imiquimod is an effective treatment for high-grade VIN and have evaluated the response and the recurrence rate with this treatment[7-9,17]. However, these researches had short follow-up periods, with median follow-up periods ranging from 5.5 to 30.5 months. About a long term follow-up period, Terlou et al[18] published a study with a median follow-up time of 7.2 years and showed that the treatment with Imiquimod is an effective long-term therapy for VIN. Most studies analyzed the different approaches to VIN and compared the outcomes of surgical and medical therapy with Imiquimod, finding that surgery remains the principal approach for VIN[10,12,19]. These results supported previous studies which also found relatively high failure and recurrence rates in patients treated with Imiquimod, compared to primary local excision[20,21]. Only one report compared single treatments and combinations ones, with contrasting findings[1]. The current work compared the outcomes of surgery and combination therapy (surgery plus Imiquimod) in terms of overall complete response and recurrence rate. The results obtained demonstrate that during 5 years follow-up, the overall complete response, the recurrence rate and the disease free time were similar in the two groups ($p = 0.7$). In our report the presence of multifocal lesions and the degree of VIN before treatment seemed to be associated to major risk of recurrence. These findings agree with those reported by other authors[22]. Smoke generally is associated with the incidence and recurrence rate of VIN[1,5], but our research did not report the association between smoke and recurrence rate, as other authors[23]. Leufflen et al[24] reported an association between multicentric disease and recurrence rate, but in our study these factors were not associated. It is common practice for oncologists to combine treatments for cancer therapy[25,26] and the most important thing for gynaecologists is to search ways to treat vulvar precancerous lesions other than surgery.

Conclusions

Although surgery remains the primary treatment for VIN, it doesn't resolve the underlying cause, a persistent HR HPV infection, for this reason adjuvant treatment such as Imiquimod therapy after surgery could be suggested. New combinated therapies don't show already significant results, so further studies about this treatment modality are needed.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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