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The re-infection rate of high-risk HPV and the recurrence rate of vulvar intraepithelial neoplasia (VIN) usual type after surgical treatment

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Antonio Frega^{1ABDEFG}, Francesco Sopracordevole^{2B}, Paolo Scirpa^{3B},
Alberto Biamonti^{4B}, Laura Lorenzon^{5GCF}, Simona Scarani^{1B}, Luana De Sanctis^{1GD},
Arianna Pacchiarotti^{1BF}, Massimo Moscarini^{1D}, Deborah French^{6ABDF}

¹ Department of Woman's Health and Territorial Medicine, Faculty of Medicine and Psychology "Sapienza" University of Rome, Rome, Italy

² Department of Gynaecologic Oncology, Aviano National Cancer Institute, Aviano (PN), Italy

³ Department of Obstetrics and Gynaecology, Catholic University, Rome, Italy

⁴ Department of Obstetrics and Gynaecology, Cristo Re Hospital, Rome, Italy

⁵ Surgical and Medical Department of Clinical Sciences, Biomedical Technologies and Translational Medicine, Faculty of Medicine and Psychology, "Sapienza" University of Rome, Rome, Italy

⁶ Department of Clinical and Molecular Medicine, Faculty of Medicine and Psychology, "Sapienza" University of Rome, Rome, Italy

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Summary

Background:

VIN usual type appears to be related to the HPV's oncogenic types. The aim of this prospective multicenter study was to evaluate the re-infection rate of high-risk HPV and the recurrence rate of VIN usual type after surgical treatment.

Material/Methods:

The study enrolled 103 women affected by VIN usual type. They underwent wide local excision by CO₂ laser. The patients were investigated by clinical evaluation and HPV DNA test 6 months after surgical treatment, and then were followed-up at 12, 18, 24, and 36 months. The recurrences were treated with re-excision.

Results:

The rate of HPV infection after surgical treatment was 34% at 6 months, 36.9% at 12 months, 40% at 18 months, 41.7% at 24 months and 44.7% at 36 months. The mean time from HPV infection to the development of VIN was 18.8 months.

Conclusions:

HPV testing in the follow-up of VIN usual type patients might be useful for identifying those patients with a higher risk of recurrence after surgical treatment, although more studies are needed. These preliminary data suggest that the test, in addition to clinical examination, can improve the efficacy of the follow-up.

key words:

VIN usual type • Human Papillomavirus (HPV) • HPV DNA test

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Author's address:

Antonio Frega, Viale Ippocrate, 97-00161 Roma, Italy, e-mail: a.frega@tin.it

BACKGROUND

The terminology for squamous vulvar intraepithelial neoplasia (VIN) was established by the International Society for the Study of Vulvar Disease (ISSVD). In this classification, abnormal changes in vulvar tissue diagnosed by cytology are categorized as VIN1, VIN2, or VIN3, but VIN1 has a low malignant potential and is not considered as a precursor to VIN 2 or 3 [1]. The most common human papilloma viruses (HPV) types in VIN1 are HPV-6 and HPV-11 [2]. Vulvar condylomata and VIN are still distinguished from one another. The reason for retaining this distinction (for the vulva) is that the vast majority of vulvar condylomata are caused by low-oncogenic-risk HPV types – HPV-6 and 11 [3,4]. The development of invasive vulvar carcinoma from a pre-existing condyloma acuminatum is an extremely rare event. Histologically, the distinction between vulvar condyloma and VIN is made on the basis of 2 features – the presence or absence of abnormal mitotic figures and the presence or absence of nuclear atypia that involve the basal and parabasal cell layers. Distinction of low-grade lesions by presence of wart-like characteristics is, however, impossible, and VIN1 has been abolished from the revised classification of VIN [1,2]. In 2003 the ISSVD proposed for vulvar intraepithelial neoplasia the term VIN for all high-grade squamous lesions. Two different categories of VIN are to be considered – VIN usual type (wart, basaloid, and mixed), is related to the HPV's oncogenic types infections, whereas VIN differentiated type is not associated with HPV and is related to lichen sclerosus and/or squamous cell hyperplasia [1]. Incident infection and risk for progression to VIN 2–3 was highest for HPV-16 [5], although HPV-18 and HPV-31 are found in vulvar disease. A meta-analysis investigated human papillomavirus (HPV) prevalence in vulvar, vaginal and anal intraepithelial neoplasia (VIN, VAIN, AIN) grades 1–3 and carcinoma from 93 studies conducted in 4 continents and using PCR assays. Overall HPV prevalence was 67.8%, 85.3% and 40.4% among 90 VIN1, 1,061 VIN2/3 and 1,873 vulvar carcinomas, respectively [2].

Until 30 years ago VIN was an uncommon condition, seen principally in middle and older age. The incidence has increased significantly since then, particularly in younger women; the median age is 35.8 years [6].

However, in contrast to carcinogenesis of CIN, the role of HPV infection in the development of VIN is still unclear; however, high-risk human papillomavirus (HR-HPV) infection, human immunodeficiency virus (HIV) infection, smoking, cervical, vaginal and anal intraepithelial neoplasia are considered to be high-risk factors for development of VIN [4].

The treatment of VIN is still debated. Surgical treatment has been proposed as the standard treatment of VIN, in order to achieve a correct staging of the disease. A wide local excision with free margins provided results as effective as vulvectomy [7]. It is difficult to treat because radical vulvar surgery for a pre-invasive lesion is inappropriate, and more conservative excisional treatment is frequently unsuccessful, particularly in cases of multifocal disease. The consequence of multiple surgical procedures can be vulvar disfigurement and loss of sexual function [8], while the objectives of treatment have expanded to include the prevention of invasive vulvar cancer and preservation of normal vulvar function and anatomy. Therefore, management options are being investigated,

including topical therapy, laser excision and vaporization, and photodynamic therapy. All can be effective in both eliminating disease and maintaining relatively normal-appearing and functioning anatomy [9]. Laser vaporization has the advantage of preservation of vulvar architecture, but recurrence rates are high [10]. Although oncogenic HPV infection is a necessary factor for development of VIN, it is likely that the persistence of high-grade lesions (VIN2/3) reflects a failure of the immune system to clear the infection, perhaps augmented by other factors. The rationale for immune-targeted therapies (topical therapy) is supported by the evidence that immunosuppressed individuals show higher rates of VIN (~30%) [8,11]. In spite of an appropriate treatment, recurrence rate may be up to 50% at 5 years, especially in young women, and the development of an invasive disease during follow-up may be up to 10 times greater than surgically treated CIN [12].

An unresolved issue in VIN's management is identification of women with a higher risk of recurrence that might require an intensive follow-up. In 1997 Kuppers demonstrated that in the prediction of possible risk of recurrence of the disease, not only the grade of VIN but also the multifocality of the lesion is important [13].

The main risk factors for recurrence, regardless of treatment used, are represented by multicentricity, multifocality, presence of high-risk HPV (HR-HPV), HIV infection and disease-free resection margins less than 5 mm [4,14,15].

Some authors advocate including the HR-HPV test in monitoring women initially treated for CIN (cervical intraepithelial neoplasia) 2–3. They suggest that all women should be tested at 6 and 24 months after treatment and only referred to the population-based cervical cancer screening program after the second negative test. They investigated the predictive value of HPV in the follow-up of patients treated for CIN2 or 3. HPV DNA testing performed 6 months after treatment was more predictive than was abnormal cervical cytology. A probable predictive value of the HPV DNA test for the monitoring of patients treated for VIN was observed [16], although the use of the HPV DNA test is not standardized in the management of the vulva.

The aim of this study was to evaluate the re-infection rate of HR-HPV and the recurrence rate of VIN usual type after surgical treatment.

MATERIAL AND METHODS

From April 2002 to March 2007, 118 patients whose histological findings revealed VIN usual type were enrolled in a prospective multicenter study.

Informed consent was obtained from all selected patients.

One hundred eighteen patients were selected according with the following inclusion criteria: 5 mm of free margins at surgery, histology consistent with VIN usual type with morphological features of viral infection (eg, presence of koilocytosis and giant cell) [5] and no persistence of disease after 3 months of follow-up.

Fifteen out of 118 patients were excluded as lost to follow-up, leaving 103 patients enrolled in this study.

Table 1. Results of follow up after treatment.

| | 6 months | 12 months | 18 months | 24 months | 36 months |
|---------------------------|------------|------------|------------|------------|------------|
| HPV + Recurrence + | 5 (4.9%) | 10 (9.7%) | 15 (14.6%) | 19 (18.5%) | 24 (23.3%) |
| HPV + Recurrence – | 30 (29.1%) | 28 (27.2%) | 26 (25.2%) | 24 (23.3%) | 22 (21.4%) |
| HPV – Recurrence – | 66 (64.1%) | 62 (60.2%) | 58 (56.3%) | 54 (52.4%) | 50 (48.5%) |
| HPV – Recurrence + | 2 (1.9%) | 3 (2.9%) | 4 (3.9%) | 6 (5.8%) | 7 (6.8%) |
| Total (n patients) | 103 | 103 | 103 | 103 | 103 |

The women underwent to colposcopy performed by means of a Zeiss 50 T colposcope (Carl Zeiss Inc.; Germany).

The histological diagnosis was assessed by a punch biopsy in the areas revealing the greatest degree of abnormalities, to differentiate VIN from condylomata, basal cell carcinoma or post-inflammatory hyperpigmentation. The clinical appearance of the lesion is not highly predictive for the presence of early stromal invasion. The samples obtained were fixed in 10% formalin and sent to the histology laboratory.

Wide local excision by CO₂ laser (Coherent System 451 instrument, by Zeiss photocolposcopy attachment) was performed in an outpatient or day-surgery setting under local anaesthesia, using 2% Carbocaine. Patients were investigated by a vulvar scraping for HR-HPV detection performed by Hybrid Capture 2 HPV DNA test (HC2; Digene Corporation, Gaithersburg, Maryland, USA) 6 months after surgical treatment, and then at scheduled follow-up at 12, 18, 24, and 36 months.

HC2 uses high-risk probes to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68.

Patients were followed-up by clinical evaluation and colposcopy, quarterly in the first year and then once a year up to the third year. Recurrences were treated with re-excision of the suspected areas and confirmed by histological diagnosis, while radical vulvectomy associated with inguinal-femoral lymphadenectomy was performed in 1 case of invasive vulvar carcinoma.

Statistical analysis

To assess the validity of the HPV DNA test in determining the possibility of recurrence, we calculated sensitivity, specificity, positive predictive value and negative predictive value. Ninety-five percent confidence intervals (95% CI) for sensitivity, specificity, positive predictive value and negative predictive value were estimated.

The mean time from HPV infection to the development of VIN was calculated.

RESULTS

A total of 103 patients affected by VIN usually type who underwent laser excision with 5 mm of free margins and no persistence of disease after 3 months of follow-up were included into this study.

Median age of selected patients was 39.3 years (range: 22–60 years). In our population study group the rate of HPV re-infection after surgical treatment was 34% (35 patients out of 103, including 5 patients with recurrence) at 6 months, 36.9% at 12 months (38 patients, in which 10 patients had recurrence), 39.8% at 18 months (41 patients, including 15 patients with recurrence), 41.8% at 24 months (43 patients, in which 18 patients had recurrence and 1 patient had invasive vulvar carcinoma) and 44.7% at 36 months (46 patients, including 24 patients with recurrence). Recurrences were observed more in patients who tested positive for HPV after surgical treatment than in those who tested negative, ranging from 6.8% (7 patients) at 6 months to 30.1% (31 patients) at 36 months, similar to what has been previously reported (50% at 60 months) [12].

According with results found in the literature, the HR-HPV test had a specificity of 0.69 (95% CI, 0.60–0.78), a sensitivity of 0.77 (95% CI, 0.69–0.85), a positive predictive value of 0.52 (95% CI, 0.42–0.62) and a negative predictive value of 0.88 (95% CI, 0.82–0.94).

The mean time from HPV re-infection to the development of VIN was 18.8 months.

Results are summarized in Table 1.

DISCUSSION

Thirty years ago a diagnosis consistent of VIN was uncommon, usually being diagnosed in middle-aged women, but its incidence has increased significantly in recent years, particularly in younger women. The median age of women at the time of diagnosis has been reported at 35.8 years [6], similar to our data (39.3 years). The increasing incidence of the condition parallels similar trends in cervical intraepithelial neoplasia (CIN) and relates at least in part to changing sexual behaviours, HPV infection, and cigarette smoking [6].

There are 2 main clinical issues related to the management of VIN – the prevention of invasive vulvar cancer and the preservation of normal vulvar anatomy and function [15].

CO₂ laser excision allows the treatment of VIN in an outpatient or day-surgery setting under local anaesthesia with excellent cosmetic and functional results. The treatment can also be adjusted to the patient's specific needs, with the possibility of calibrating the depth of vaporized and removed tissues. Excisional treatment is the gold standard method since it allows the histological evaluation of the excised tissue [17], disclosing the complete removal of the lesion and the exclusion of an early invasive disease. Although there are no clear

recommendations regarding the size of margins, most authors believe that 5 mm of free margin is adequate for VIN [15].

We reported a recurrence rate of 30.1%, of which 23.3% were HPV DNA-positive, and we closely follow-up recurrences with local excision of suspicious vulvar lesion. One case of invasive vulvar carcinoma was observed during follow-up, and it was adequately treated. The definition of failure of treatment may be difficult in VIN treatment. The difference between local persistence or relapse versus the development of new lesions cannot always be distinguished retrospectively [15].

HPV DNA testing before the treatment does not predict the quality of loop excision, as in CIN lesions, the risk of persistence, or the recurrence of disease. Post-treatment HPV DNA test have a good negative predictive value and thus could be useful for monitoring patients after loop excision. If a post-treatment HPV DNA test discloses negative results and if the margins are free, the follow-up could be reduced [18]. Even though we evaluated VIN patients, it seems reasonable that same clinical approach could be used for patients with VIN. HR-HPV infection clearance after conization, with clear resection margins, has been reported in ~93% of patients at 6-month follow-up [19], while HR-HPV infection clearance after surgical treatment of VIN, with clear resection margins, has been reported in 66% of patients at 6-month follow-up.

HPV-16 is a predictor for persistent HPV after loop excision in patients with negative margins [20]. This could explain the high rate of HPV-positive status after surgical treatment of VIN at 6 months, probably caused by presence of HPV-16.

A study from the U.S. found the median time from the first detection of an HPV infection to the development of CIN 2-3 was 14.1 months [16,21], similar to our data (18.8 months); indeed, the median time from vulvar HPV infection to VIN might be comparable to that of CIN.

Our study was conducted on women enrolled in different medical institutions, whereas most of the other studies reported to date examined the distribution of HPV types in VIN lesions in a single region or medical institution [16,22–26].

Past studies reported a high risk for progression to VIN 2-3 for HPV-16 [5], although HPV-18 and 31 could also be found in vulvar diseases. Prophylactic vaccination against HR-HPV will likely become an important tool for VIN prevention [7], even though HPV-31 is a HPV type not included in the vaccination assay and this may require close observation of patients.

CONCLUSIONS

HPV DNA testing in the follow-up of VIN usual type patients might be useful for identifying those patients with a higher risk of recurrence after surgery, although more studies are needed. These preliminary data suggest that HPV-DNA testing, in addition to clinical examination, could improve the efficacy of follow-up in patients treated for VIN usual type.

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