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Vulvar cancer: Two pathways with different localization and prognosis

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HIGHLIGHTS

- The $p16^{INK4A}$ expression should take leading role in determining HPV-relation.
- HPV-related vulvar SCC has better prognosis and perineum as predilection site.
- HPV-related vulvar SCC is a separate entity and other treatment modalities need to be investigated.

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ABSTRACT

Background. Two etiologic pathways for vulvar squamous cell carcinoma (SCC) are described: in a background of lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia and related to high-risk human papillomavirus (HPV) infection with high grade squamous intraepithelial lesion (HSIL) as precursor. The aim was to compare the predilection site and survival of HPV-related to non HPV-related vulvar SCCs.

Methods. Data of patients treated for primary vulvar SCC at the Radboudumc between March 1988 and January 2015 were analyzed. All histological specimens were tested for HPV with the SPF_{10}/DEIA/$LiPA_{25}$ system assay and $p16^{INK4a}$ staining was performed using CINtec® histology kit. Vulvar SCCs were considered HPV-related in case of either >25% $p16^{INK4a}$ expression and HPV positivity or >25% $p16^{INK4a}$ expression and HSIL next to the tumor without HPV positivity. Tumor localization, disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) of patients with HPV-related and non HPV-related vulvar SCC were compared.

Results. In total 318 patients were included: 55 (17%) had HPV-related (Group 1) and 263 (83%) had non HPV-related vulvar SCC (Group 2). Tumors in Group 1 were significantly more often located at the perineum compared to Group 2, 30% and 14%, respectively ($p = 0.001$). The DSS, DFS and OS were significantly better in HPV-related than in non HPV-related vulvar SCC patients.

Conclusion. HPV-related vulvar SCCs are more frequently located at the perineum and have a favorable prognosis compared to non HPV-related vulvar SCCs. Both localization and HPV-relation could explain this favorable prognosis. HPV-related vulvar SCC seems to be a separate entity.

1. Introduction

Vulvar cancer is a rare disease, representing approximately 3–5% of the malignancies of the female genital tract [1], but with an increasing incidence rate from 2002 onwards [2]. Around 80% of the malignant tumors of the vulva are squamous cell carcinomas (SCCs). Most SCCs of the vulva occur on the labia majora, but the labia minora, clitoris and perineum may also be primary sites.

There are two different etiologic pathways for the development of vulvar SCC. The first pathway is the most common pathway, with its precursor lesion differentiated vulvar intraepithelial neoplasia (dVIN) and often occurs in the background of lichen sclerosus (LS). The second pathway is related to the high-risk human papillomavirus (hrHPV) infection and covers about 25–30% of all vulvar SCCs [3]. High grade squamous intraepithelial lesion (HSIL) of the vulva, formerly known as usual vulvar intraepithelial neoplasia, is the precursor lesion [4]. Differentiated VIN is suggested to be highly proliferative and therefore more likely to progress to vulvar SCC compared to HSIL [5]. Remarkably, most vulvar SCCs are dVIN-related but most isolated premalignant lesions are HSILs [3]. In general, the possibility of HSIL progressing into

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vulvar SCC is low in comparison to dVIN [6], namely 9–16% in untreated patients and 3% in treated patients, compared to about 30% in case of dVIN [7].

The role of HPV in cervical cancer is clearly established: an infection with hrHPV is the most important etiological step in the pathogenesis of cervical carcinoma [8]. Most cervical (pre)malignancies occur at the transformation zone: an area where squamous epithelium merges into columnar epithelium. The cervical transformation zone is most susceptible for an HPV infection because of the high cell turnover and close relation to the basal cell layer [9]. The anus also has a similar transformation zone where hrHPV associated anal intraepithelial lesions (AIN) and anal cancer occur. The incidence of vulvar SCC is similar to that of anal cancer, however only a minority of all vulvar SCCs is caused by hrHPV infection. Nevertheless, the oncogenic pathway of how HPV causes vulva SCC remains unclear. Our hypothesis states that microtraumata, due to, for example, friction caused by sexual intercourse, might facilitate access of HPV to the basal cell layer, may result in integration and finally cause vulvar SCC. A previous study by our study group investigated the influence of localization on the prognosis of vulvar SCC. Tumors with clitoral involvement had worse prognosis compared to other localizations on the vulva. Several tumor characteristics such as depth of invasion were less favorable and these characteristics were more often present in vulvar SCC located at the clitoris [10].

Several studies have evaluated the relationship between HPV infection and disease specific survival (DSS) in vulvar SCC. These studies showed contradictory results. The studies of Monk et al. [11] and Ansink et al. [12] suggest that HPV-positive vulvar SCC (established by HPV PCR only) has a better DSS, where patients with HPV negative vulvar SCC have an increased risk of recurrence and death from vulvar SCC. Van de Nieuwenhof et al. found dVIN-associated vulvar SCC to have a significantly worse DSS [13]. However, Alonso et al. [14] retrospectively evaluated 98 patients and found HPV-positive and negative vulvar SCCs to have a similar overall survival and disease free survival. Furthermore, Pinto et al. [15] reported HPV status in the tumor as not important in terms of prognosis. Two other studies found that not HPV-positivity, but the overexpression of $p16^{INK4A}$ might be associated with higher survival rates and better prognosis in vulvar SCC [16,17].

The aim of the current study was to investigate the predilection site of HPV-related compared to non HPV-related vulvar SCC to better understand the oncogenesis of HPV-related vulvar SCC. Secondly, we assessed the disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) in patients with HPV-related and non HPV-related vulvar SCC.

2. Material & methods

2.1. Patients and data

Data of all consecutive patients diagnosed with vulvar SCC who were primarily surgically treated at the Department of Gynecologic Oncology of the Radboud university medical center, The Netherlands, were prospectively collected and stored in our local vulvar SCC database. Data were collected by consulting the medical files and pathology reports of the patients (N = 520). For the current study we used data of patients who have been treated between March 1988 and January 2015. In total 232 patients were treated between 1988 and 2006; we used the data of 130 patients, because these data were available and complete [13]. From 2006 on, all vulvar SCC patients were included. All data were anonymously processed so no ethical approval was necessary.

Parameters extracted from the database included: patient characteristics (age at diagnosis, history of tobacco use, immune status, history of LS), histopathological characteristics (localization of the tumor, tumor diameter, depth of invasion, presence of dVIN or HSIL, type of VIN, focality, lymphovascular space invasion (LVSI), differentiation grade, presence of positive groin nodes, extra nodular growth in positive lymph nodes, and International Federation of Gynecology and Obstetrics (FIGO) stage 2009), treatment characteristics (adjuvant treatment, recurrences, and cause of death) and survival (DSS, DFS, and OS). The localization of the tumor was divided in four groups: clitoris, labium, perineum, and both clitoral and perineal involvement.

2.2. Histology

All histological specimens were collected from the archives of the department of Pathology at the Radboudumc. All slides were evaluated by two pathologists (JB and PB) on the presence of vulvar SCC. The histological specimens which contained vulvar SCC were used to determine the presence of HPV and to assess $p16^{INK4a}$ expression. The above mentioned pathologists also interpreted the degree of $p16^{INK4a}$ expression.

2.3. HPV presence and genotyping

DNA was isolated from formalin fixed paraffin-embedded tissue sections (4 μm) with 250 μl Proteinase K solution and incubated overnight at 70 °C. This was followed by heat inactivation of Proteinase K at 95 °C for 10 min. RNaseP/IC qPCR was used to evaluate DNA quality and PCR inhibition [18]. Each isolation and PCR run contained HPV positive and HPV negative controls. Specimens were tested for HPV DNA by PCR amplification/detection/typing using the HPV SPF_{10} PCR-DEIA-$LiPA_{25}$ version 1 assay (Labo Bio-medical Products, Rijswijk, The Netherlands). Briefly, the broad-spectrum HPV SPF10 PCR amplifies a 65 bp fragment of the L1 open reading frame and recognizing a broad spectrum of at least 65 different HPV genotypes. SPF_{10} DEIA was used for detection of generated amplimers. All SPF_{10} PCR DEIA-positive samples were used for subsequent genotyping by reverse hybridisation line probe assay (LiPA), allowing simultaneous typing of the 25 mucosal HPV types (HPV6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70 and 74). The combined HPV SPF_{10} PCR-DEIA-$LiPA_{25}$ system for detection and genotyping of HPV has been described in detail elsewhere [19–21]. Only the hrHPV positive vulvar SCC samples were considered HPV positive.

2.4. $P16^{INK4A}$ immunohistochemical expression

Tissue sections (4 μm) of the archival paraffin-embedded tissue samples of vulvar SCC were mounted onto SuperFrost glass slides (Menzel-Gläser, Braunschweich, Germany) and dried overnight at 37 °C.

In total 379 samples were stained. One hundred and thirty samples were stained earlier in light of previous conducted research [13]. Immunohistochemistry for $p16^{INK4a}$ of the remaining 249 samples was performed using the automated Ventana XT system (Ventana Medical Systems, Inc., Tucson, AZ). The reaction was developed using CINtec® p16 histology kit (Ventana Medical Systems, Inc.). All sections were then counterstained with hematoxylin, dehydrated, and cover-slipped.

Interpretation of $p16^{INK4A}$: nuclear and cytoplasmic $p16^{INK4A}$ staining were both considered as a positive reaction. The results were reported in a semi quantitative fashion: negative (−) if <5% of the cells had nuclear or cytoplasmic staining, slightly positive (1+) if 5 to 25% of the cells were stained, moderately positive (2+) if staining was present in 25 to 75% of the cells, and markedly positive (3+) if >75% of the cells showed nuclear or cytoplasmic staining.

2.5. HPV-related and non HPV-related

Based on the existing literature on HPV-related malignancies, we believe that $p16^{INK4A}$ expression in the tumor is essential in the subdivision of HPV-related and non HPV-related vulvar SCC. $P16^{INK4A}$ expression is a surrogate marker of HPV infection. HPV infection leads to the inhibition of retinoblastoma protein pRB via HPV E7 protein which then leads to increased $p16^{INK4A}$ expression through a feedback

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