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Case report

A case of human papillomavirus infection and vulvar cancer in a young patient – "hit and run" theory

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ABSTRACT

Vulvar cancer (VC) is a rare disease, of which the squamous vulvar carcinomas (SVCs) are the most common histological subtype. SVC is often associated with human papillomavirus (HPV) infection. HPV- positive SVCs are multifocal, typically have non-keratinizing morphology, presence of koilocytes and tend to arise in younger women (<50 years), which are often smokers. The "hit and run" theory has been a subject of longstanding curiosity in tumor virology. The "hit and run" scenario suggests that viruses have an activating role in the cancer development and the viral genome may disappear after the host cell accumulates numerous mutations.

Herein, a case of HPV- positive SVC in a 22-year-old patient with a possible "hit and run" scenario, is presented. Gynecological examination revealed a vulvar mass (3 cm) with ulcerated surface, located at the left Bartholini gland area. Punch biopsies of the lesion were performed. The histopathological examination revealed non-keratinizing squamous cell carcinoma (Grade 2) of the vulva and presence of koilocytes. P16 immunostaining was block-positive. HPV-testing of the specimen was negative.

In the majority of cases, VC arising in young patients is associated with HPV. VC located in the BG area should be distinguished from BG carcinoma. Future studies should reconsider the third diagnostic (histological areas of apparent transition from normal elements to malignant ones) criteria for defining BG carcinoma. The "hit and run" theory is rarely mentioned in oncology, but should be considered in cancer- associated viruses. The "hit and run" affair suggests that viruses may cause more cancers than previously thought.

1. Introduction

Vulvar cancer (VC) is a rare disease, accounting approximately 4% of all gynecological malignancies (Mitra et al., 2018). Squamous cell vulvar carcinoma (SVC) is the most common histological subtype of all vulvar malignancies (Cheng et al., 2016). It constitutes about 80% of VC cases, of which 9 to 70% are due to human papillomavirus (HPV) (Mitra et al., 2018, Cheng et al., 2016, Hajeer et al., 2020). Iwasaka et al. observed on Syrian hamster embryo cells the 'Hit and run' oncogenesis by human papillomavirus type 18 DNA (Iwasaka et al.,1992). It has been established that vulvar intraepithelial neoplasia and HPV are the dominant pathways of VC occurrence in young women (Hajeer et al., 2020). HPV is double-stranded DNA virus, which belongs to the Papillomaviridae family and it is classified into 5 groups: alpha, beta, gamma, mu, and nu (Mitra et al., 2018, Ferreira et al., 2021, Rollison et al., 2019). Over 200 types have been described of which HPV 16 and 33 subtypes are predominant and account for 55.5% of all HPV-related vulvar cancers (Mitra et al., 2018, Ferreira et al., 2021). Moreover, recently VC is separated biologically and clinically as distinct two phenotypes - HPV-positive tumors and HPV – independent tumors. HPV-

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positive tumors have a better prognosis as chemotherapy and radiotherapy significantly impact overall survival (Allo et al., 2020). The "hit and run" theory has been a subject of longstanding curiosity in tumor virology (Niller et al., 2011). The "hit and run" scenario suggests that viruses have an activating role in cancer development and the viral genome may disappear after the host cell accumulates numerous mutations (Ferreira et al., 2021). Herein, a case of HPV- positive SVC in a young patient with a possible "hit and run" scenario, is presented.

2. Case report

A 22-year-old ethnic minority female patient (gravida 1, para −1) admitted to the department of gynecology with a shot history of fever – 38.5 °C, fatigue, edema and pain located on the left labia majora, at the left Bartholini gland area. The patient had no major gynecological or medical diseases and no family cancer history in first-degree relatives. The patient has had history of tobacco use for 5 years. She had one vaginal delivery (without complications) 6 years ago. The patient last menstruation was 20 days ago. Gynecological and ultrasound examination were without abnormalities except for a vulvar mass (3 cm) with ulcerated surface, located at the left Bartholin gland area. The lesion was tender, painful and seemed to infiltrate the underlying tissues. The vagina and cervix were without macroscopic abnormalities. Pap test, cervical and vaginal colposcopy were not perfomred after the biopsy, as the patient refused. Laboratory examinations were without abnormalities. The computed tomography revealed no distant metastasis. Punch biopsies of the lesion were performed. The histopathological examination revealed non-keratinizing squamous cell carcinoma of the vulva (Grade 2). As the pathologist observed koilocytes in the biopsy specimen, p16 immunostaining was performed to determine the HPV status of the tumor. Block-positive cytoplasmic and nuclear p16 immunostaining were noticed (Figs. 1, 2). HPV Polymerase Chain Reaction (PCR/Real time) was carried out for the following alpha HPV subtypes – high- risk HPV – 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66/68, intermediate risk HPV – 53, 73, 81, 82, low-risk HPV – 6, 11, 40/61, 42, 43/44, 54/55, 70, 57/71, 72, 84/26. PCR was negative for all HPV subtypes.

The patient was referred to another health care institution where a radical left hemivuvlectomy with ipsilateral inguinal lymph node dissection were performed. The histopathological examination revealed non-keratinizing squamous cell carcinoma of the vulva and two lymph nodes showed metastatic tumor. The patients was staged IIIB according to FIGO classification and T1bN1M0 according to TNM classification. The woman was referred to adjuvant radiation therapy.

3. Discussion

The present case raises many debates and discussions. Initially, we believed that the lesion is a Bartholini gland (BG) carcinoma. The patient was young, the lesion was located in the BG region and HPV was suspected histologically (koilocytes were identified). The diagnostic criterias of the BG carcinoma include: 1 – the tumor has correct anatomic position (deep in the labium majus) and it is histologically consistent with BG carcinoma; 2- no evidence of concurrent primary tumor; 3- histological areas of apparent benign to malignant transition. The present case covers most of the diagnostic criterias, except for the third one - histological areas of apparent benign to malignant transition. Some authors stated that such tumors without benign to malignant transition represented probable BG carcinomas, as there were cases with complete tumor replacement of the gland. Although in the present case the patient had probably had BG carcinoma, we used the widely accepted criteria and concluded that vulvar cancer was the final diagnosis. However, we believe that revision of diagnostic criteria for BG carcinoma should be considered in future studies (Cardosi et al., 2001).

The association between some viruses and cancers have been well established. Viruses such as Epstein–Barr virus, human herpesvirus 8, also called Kaposi's sarcoma-associated herpesvirus, hepatitis B and C viruses, Merkel cell polyomavirus, human T-cell leukemia virus type 1virus; human papillomaviruses (HPVs) are distinctly recognizable as human tumor viruses. They are responsible for approximately 12% of all human cancers (Ferreira et al., 2021, Niller et al., 2011). HPVs are responsible for the majority of cervical, vaginal and vulvar cancers. HPVs are divided into three types – low-risk HPV (most common 6, 11), intermediate-risk HPV (53, 73, 82) and high-risk HPV (most common −16, 18, 31, 33) (Mitra el al., 2018, Cheng et al., 2016, Hajeer et al., 2020). HPV- positive SVC are multifocal, typically have non-keratinizing morphology, presence of koilocytes and tend to arise in younger women (<50 years), which are often smokers. In the majority of cases, the HPV-

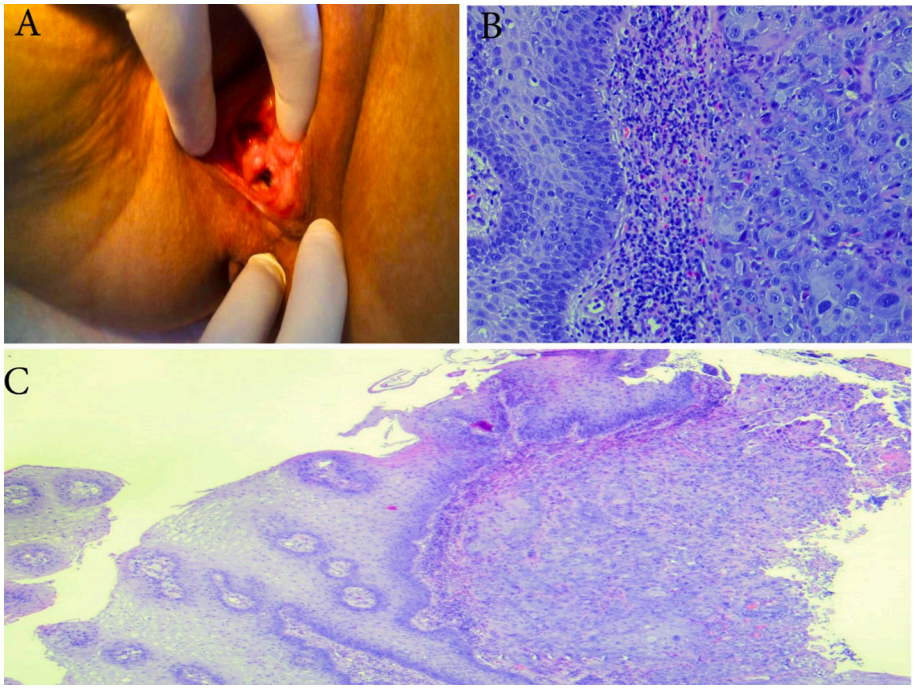

Fig. 1. Macroscopic and histological appearance of VSC in the present case. A – Ulcerated lesion located on the left labia major, at the left Bartholini gland region. B - On the left preserved multilayered squamous epithelium, on the right infiltration of tumor atypical squamous cells, lymphoid stroma between them (HEx200). C - Non-keratinizing multilayered squamous epithelium with acanthosis, papillomatosis, focal koilocytic atypia with a focus of low-grade dysplasia at the periphery with underlying infiltration from nests of atypical squamous cells with moderate lymphocyte stromal response (HEx40).

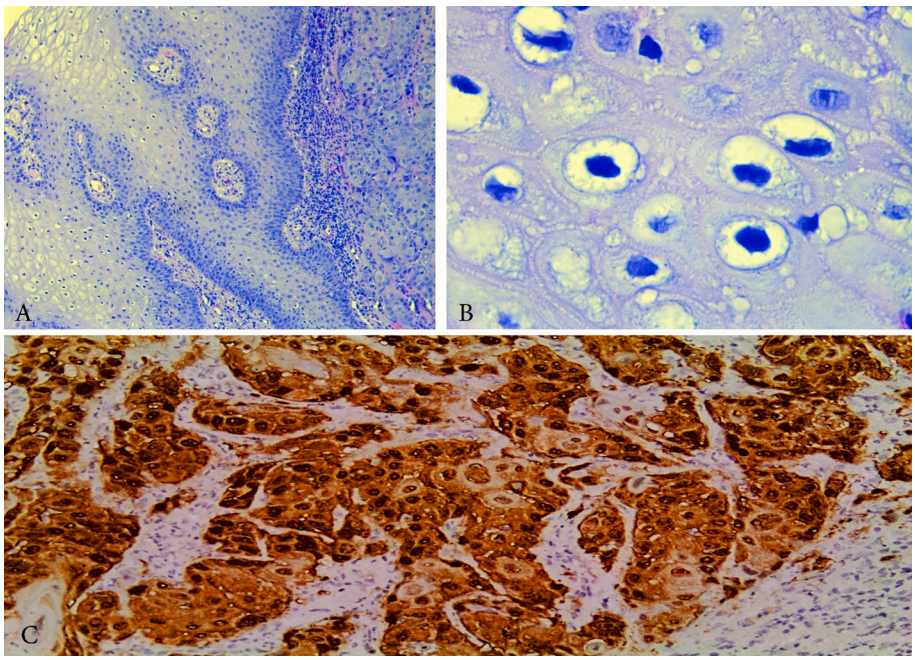

Fig. 2. Histological and immunohistochemical appearance of VSC in the present case. A- On the left preserved multilayered squamous epithelium with prominent koilocytic atypia, on the right infiltration of tumor atypical squamous cells, lymphoid stroma between them (HEx100) B - Koilocytic atypia in squamous epithelium (HEx400). C – Block-positive p16 immunoreactivity in the tumor nests..

positive SCV histomorphologicaly is associated with warty or basaloid vulvar intraepithelial neoplasia (Allo et al., 2020). HPV-negative tumors are usually unifocal, affect elder women, typically have a low-grade keratinizing morphology, lack of koilocytes, and are often associated with dysplasia simplex and lichen sclerosis (Hajeer et al., 2020).

The double-stranded DNA genome of the HPV consists of three segments – long control region (LCR – 10% of the genome), early genes (50%) and late genes (40%). The late genes encode two late proteins (L1, L2), whereas the early genes produce six early proteins (E1, E2, E4, E5, E6, E7) (Lee et al., 2016). HPV oncogenes associated with cellular transformation are E6 (degrades tumor suppressor p53) and E7 (inhibits tumor suppressor pRb) (Ferreira et al. 2021, Lee et al., 2016). HPV initiates to the basal epithelial layer through micro-lesion of the tissues and activates the late viral genes, which produce capsid protein formation and viral DNA replication. Later, after integration of HPV DNA into the genome the deletion of many early (E1, E2, E4, and E5) and late

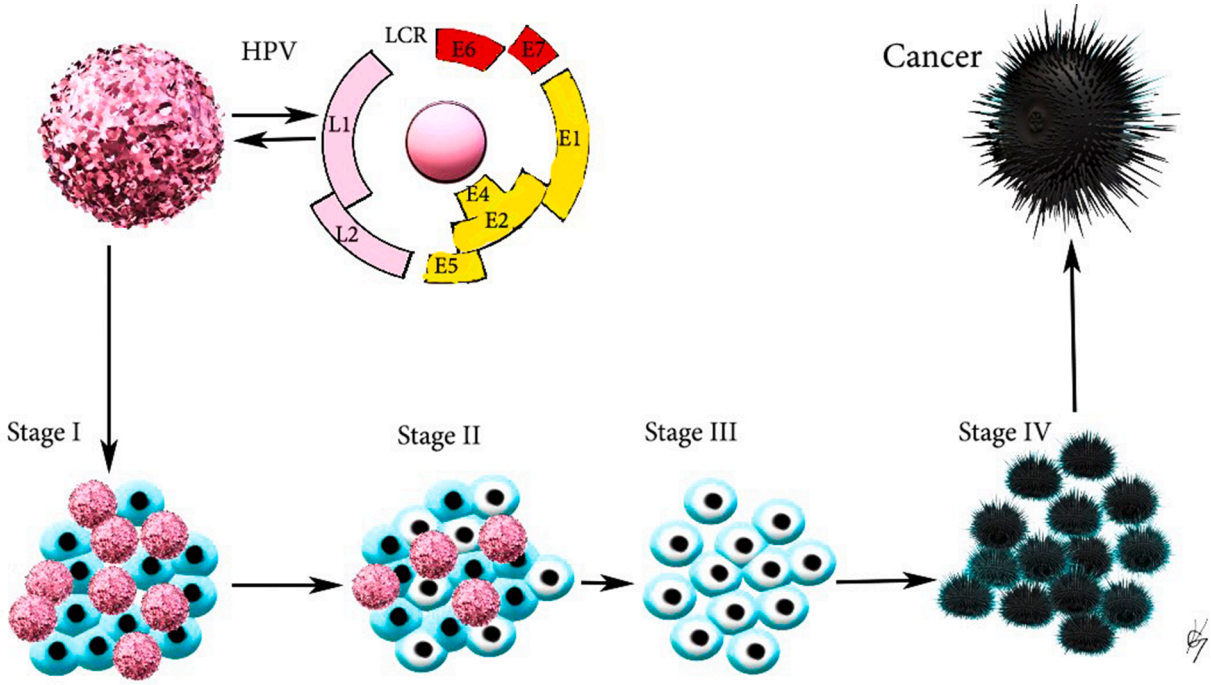

Fig. 3. HPV infection and "hit and run" affair. **Stage I** – HPV invasion through micro lesions of the tissues and cells. **Stage II -** integration of HPV DNA into the genome in the E1/E2 region of the infected cell, accumulation of mutations in the host cell. **Stage III -** the HPV genome is entirely lost after the host cell accumulates numerous mutations. **Stage IV –** cancer development.

(L1 and L2) genes occurs. The absence of E2 leads to elevated expression of oncoproteins E6 and E7 and consequently the ability to progress to cancer (Ferreira et al. 2021, Lee et al., 2016). The main idea of "hit and run" theory in HPV is that after expression of oncoproteins E6 and E7, the development of cancer is already started and mutations are accumulated over time through carcinogenesis. Consequently, HPV is not necessary anymore for the maintenance of the cancer. Therefore, after initiating a heritable change in the gene expression pattern of the host cell, the genomes of HPVs can be completely lost (Fig. 3) (Niller et al., 2011).

In the present case, the patient had histopathological sings of HPV infection – p16 block-positive immunostaining in neoplastic squamous epithelium and presence of koilocytes. However, there was discordance between PCR and p16 immunostaining. Cheng et al. reported 3 cases among 22 patient, of discordance between PCR HPV testing and p16 immunostaining (Cheng et al.,2016). In the present article we concluded that there were four possible scenarios - false-negative PCR, damaged of viral DNA after formalin fixation, the presence of an HPV genotype not detected by the PCR primers (the patient was not tested for beta HPV) or "hit and run" scenario. Studies suggest that beta HPV and ultraviolet radiation are promoters of non-melanoma cutaneous squamous cell carcinoma (Rollison et al., 2019). Recent studies separated HPV-independent and HPV- related cancers. HPV -related cancers are sensitive to chemotherapy, radiotherapy and are associated with better prognosis. Therefore, it is of importance if p16 immunostaining is enough for accurate classification of these two types. Moreover, p16 could be detected after possible "hit and run" scenario as immunostaining for p16 detects a protein, which is overexpressed in host cells after infection by the oncogenic HPV (Cheng et al., 2016, Allo et al., 2020). Cheng et al. studied 50 SVCs, which showed features suggestive of HPV-associated, and 47 of those showed p16 immunoreactivity (94% concordance). Authors concluded that p16 immunostaining is an accurate marker for determination of HPV status in SVC (Cheng et al., 2016).

4. Conclusion

In the majority of cases, vulvar cancer arising in young patients is associated with HPV. VC located in the BG area should be distinguished from BG carcinoma. The "hit and run" theory is rarely mentioned in oncology, but should be considered in cancer- associated viruses. The "hit and run" affair suggests that viruses might cause more cancers than previously thought.

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CRediT authorship contribution statement

Stoyan Kostov: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft. **Deyan Dzhenkov:** Investigation, Resources, Writing - review & editing, Visualization. **Dimitar Metodiev:** Methodology, Investigation, Data curation. **Yavor Kornovski:** Formal analysis, Writing - review & editing, Visualization. **Stanislav Slavchev:** Formal analysis, Data curation, Writing - review & editing. **Yonka Ivanova:** Methodology, Formal analysis, Resources, Data curation, Writing - review & editing. **Angel Yordanov:** Methodology, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Authors declare that all related data are available concerning researchers by the corresponding author's email.

Ethical approval

This is a case report and it didn't require ethical approval from ethics committee according to our institution.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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