A 26-year-old Hispanic woman complaining of “itching” and “herpetic lesions” on the vulva for 9 months was seen at a university hospital. On physical examination, multiple vulvar masses were noted. Biopsies taken from these lesions showed invasive keratinizing squamous cell carcinoma. The vulvectomy specimen revealed 4 tumor masses, the largest located on the mons pubis. Although the incidence of vulvar intraepithelial neoplasia has increased in recent years, only very few cases of invasive carcinoma have been reported in young women. The tumors that occur at a younger age characteristically have basaloid or warty histology, in contrast to those occurring in older women, which usually are well-differentiated keratinizing carcinomas. We believe this is an unusual case of vulvar squamous cell carcinoma. In addition to our patient’s young age, her tumor had a histologic profile usually found in lesions of an elderly woman. The tumor was negative for human papillomavirus by polymerase chain reaction analysis and was positive for p53 by immunohistochemistry. (Arch Pathol Lab Med. 2001;125:267–270)

REPORT OF A CASE

A 26-year-old woman, gravida 4 para 3, presented to the Gynecology Clinic of the University of Texas Health Science Center at San Antonio complaining of “itching” and “herpetic lesions” on the vulva for 9 months. On physical examination, a total of 4 vulvar lesions was noted. The inguinal lymph nodes were palpably enlarged. The differential diagnosis at admission included lymphogranuloma venereum and invasive SCC. Biopsies taken from the vulvar lesions revealed well-differentiated keratinizing invasive SCC. The patient underwent radical vulvectomy and excision of bilateral ileoinguinal lymph nodes.

PATHOLOGIC FINDINGS

Gross examination revealed 3 exophytic and 1 ulcerated lesion (Figure 1). The lesions were located on both labia majora and the mons pubis. The largest lesion was located in the mons pubis and measured 5.5 cm in diameter by 1.5 cm in thickness. On sectioning, this lesion extended into the underlying subcutaneous adipose tissue. Histologic examination of all 4 lesions showed well-differentiated KSC (Figure 2) with a positive superior margin at the level of the mons pubis. The clitoris itself was free of tumor; however, the tumor extended into the erectile tissue of the left labium minus. Vulvar intraepithelial neoplasia (VIN) of the usual basaloid type or HPV changes were not seen. The adjacent vulvar epithelium not involved by invasive carcinoma showed extensive squamous hyperplasia (Figure 3) and focally SCC in situ of the simplex type (Figure 4). Metastatic carcinoma was found in 4 of 15 left, and 1 of 3 right ileoinguinal lymph nodes. On the right side, extracapsular extension was noted.

Immunohistochemistry for p53 (DO7, Novocastra, Burlingame, Calif) was performed using the streptavidin–horseradish method. The tumor cells in both the invasive and in the situ component were immunoreactive for p53 (Figure 5). The adjacent areas of squamous hyperplasia showed no immunoreactivity with p53. Polymerase chain reaction (PCR) for HPV detection was carried out as described previously with minor modifications. The primers used were the extensively characterized MY09-MY11 primer set. This system is capable of detecting greater than 40 mucosotropic HPV types. DNA amplifiability was tested by using a beta-globin–specific primer set, which generated a 166-base-pair product. Sections of formalin-fixed, paraffin-embedded CaSki and HeLa cells were used as HPV-16– and HPV-18–positive controls, respectively. In addition, fresh purified DNA from unprocessed CaSki cells was also used at an empirically determined limiting dilution as a low positive control. Negative controls included all reaction components without DNA blanks, as

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well as the use of a HPV-negative control lymphocyte cell line. For the patient, 2 separate blocks of the biopsy and 1 block from the vulvectomy selected under microscopic control were evaluated. Hence, the tumor was evaluated in triplicate; the volume of tumor in the selected sections exceeded the volume of cancer cells in the cell line controls. Rigorous anticontamination procedures, including physical separation of the pre- and post-PCR procedures were followed throughout. Five-micrometer sections were sterilely placed into a 1.5-mL tube. The sample was serially extracted with xylene and ethanol to remove the paraffin, and then the DNA was released via proteinase K digestion (60 μg/mL; Oncor, Purchase, NY) at 55°C for 2 hours, followed by boiling for 20 minutes. Five percent of

Figure 1. Gross specimen, radical vulvectomy with 4 tumors. The largest mass (5.5 × 3.5 × 1.5 cm) occupied most of the mons pubis (large arrow). The smaller mass (2.5 × 2.0 × 0.5 cm) was lateral to the clitoris (small arrow). An ulcerated mass (1.5 × 2.0 × 0.5 cm) was located on the left labia majus (large arrow head). Another ulcerated mass (3.5 × 2.0 × 0.5 cm) was located on the right labia majus (small arrow head).

Figure 2. Invasive squamous cell carcinoma, keratinizing type (hematoxylin-eosin, original magnification ×400).

Figure 3. Squamous hyperplasia adjacent to invasive tumor (hematoxylin-eosin, original magnification ×200).

Figure 4. Detail of carcinoma in situ of the simplex type showing nuclear pleomorphism and mitotic activity in the basal layer (hematoxylin-eosin, original magnification ×400).

Figure 5. Positive immunostaining in tumor for p53 (immunoperoxidase, ×400).
this 100-μL extract was subjected to PCR in a 50-μL final reaction volume. Each amplification mixture had a final concentration of 10 mmol/L Tris-hydrochloric acid, pH 8.3, 50 mmol/L potassium chloride, 3.5 mmol/L magnesium chloride, and 200 μmol/L deoxyribonucleic phosphates. Beta-globin primers had a final concentration of 0.04 μmol/L; all other primers were at 0.1 μmol/L. Hot start PCR was accomplished using Amplitaq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn) with a 13-minute activation step at 94°C. The concentration of magnesium chloride was controlled using Hot wax MgCl₂-containing wax beads (Invitrogen, Carlsbad, Calif.). A Perkin-Elmer 9600 thermal cycler was used for 50 cycles of PCR with 30-second denaturation, annealing, and elongation steps of 94°C, 55°C, and 72°C, respectively, with a terminal 10-minute elongation at 72°C. After amplification, 10 μL (20%) of the product was size-fractionated via electrophoresis on a 3% agarose gel in TBE buffer. The reactions were evaluated following staining in 10 μL/mL ethidium bromide under ultraviolet light using an Alpha Innotech imaging system (San Leandro, Calif).

COMMENT

The incidence of VIN has markedly increased in recent years. The incidence of VIN has markedly increased in recent years. In addition, VIN is becoming more common in young women between 20 and 35 years of age. These lesions frequently coexist with HPV infection and are multifocal in distribution. Despite this increased incidence, the diagnosis of invasive SCC in young women is still rare. For instance, invasive SCC in women between the ages of 23 and 35 years comprised only 3.3% of patients with malignancies of the vulva seen at the University of Michigan Hospital during a 30-year period. Roman and collaborators reported three additional patients between the ages of 16 and 25 years with invasive SCC of the vulva presenting as a unifocal lesion. Their second case is histologically similar to ours in that their photomicrograph appears to show a well-differentiated KSC. These 3 cases were somewhat aggressive, since at the time of diagnosis, all 3 carcinomas were stage T2 and had significant degrees of invasion.

Vulvar SCC has been divided into 2 different groups based on morphologic features and HPV status. Keratinizing squamous cell carcinoma is the most common type and is mainly seen in older women. In contrast, young women usually have tumors with basaloid or warty histology. Changes seen in the adjacent epithelium are also different. Basaloid and warty tumors have associated VIN III with histologic features similar to the invasive component, basaloid and warty type, respectively, or a mixture of both. In contrast, the women with KSC have either no abnormalities or vulvar dystrophies, such as lichen sclerosus or squamous hyperplasia. Carcinoma in situ of the simplex type is also present. Kim and collaborators identified the presence of p53 mutations in HPV-negative vulvar SCC. Although our patient did not have associated lichen sclerosus, a small focus of SCC in situ of the simplex type close to the invasive component overexpressed p53. The adjacent squamous hyperplasia was negative for p53.

In conclusion, several findings made this case of vulvar SCC somewhat unusual. This patient was only 26 years old, which is far below the mean age for this type of carcinoma. Although the incidence of VIN has increased in recent years, there have been only a few reported cases of invasive SCC in such young women. In addition, young women usually have basaloid or warty-type tumors, which are associated with HPV. In contrast, our patient had the type of tumor seen in the older women, and it was HPV negative by PCR studies. Overexpression of p53 was observed both in the invasive tumor and the associated simplex-type carcinoma in situ, but not in the adjacent squamous hyperplasia. The cause of this tumor in such a young woman is not completely clear. Carcinogenesis is a multistep process that involves multiple host and environmental factors. Although we ruled out HPV infection, a well-known cause of vulvar carcinoma, there are probably additional unknown environmental factors that allowed abnormal cells in this patient to escape immune surveillance. Our patient was also tested for HIV infection. Although this test was negative, she might have an unknown immune deficiency that allowed abnormal cells to proliferate and progress to cancer.

References

5. Carlson et al. 16 have recently reported p53 overexpression in areas of lichen sclerosus, suggesting that p53 mutations are involved early in the pathogenesis of HPV-negative vulvar SCC. Although our patient did not have associated lichen sclerosus, a small focus of SCC in situ of the simplex type close to the invasive component overexpressed p53. The adjacent squamous hyperplasia was negative for p53.


