Verrucous Carcinoma of the Cervix: Detection of Carcinogenetic Human Papillomavirus Types and their Role during Follow-up

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Abstract. Background: Verrucous carcinoma is a rare variant of well-differentiated squamous cell carcinoma of the uterine cervix. Infection with high-risk human papillomavirus (HPV) is the main cause of intraepithelial and invasive neoplasias of the female genital tract, but the aetiology of verrucous carcinoma is still unknown. The aim of this study was to evaluate the presence of HPV in verrucous carcinomas of the cervix and to investigate the persistence and the role of high risk HPV types in follow-up. Patients and Methods: Three patients, with atypical cytologies, were observed. All the patients underwent cytology and colposcopy followed by direct biopsy. The Hybrid Capture II microplate method was used for molecular detection of pre-surgical low- and high-risk HPV types. HPV tests were performed during the follow-up, annually. Results: The cytological findings revealed that atypical squamous cells “cannot rule out high-grade lesion” (ASC-H) in one case and high grade squamous intraepithelial lesion (H-SIL) in two cases. The HPV test detected high risk HPV types. Colposcopic findings showed cervical exophytic lesions. The histology revealed a well-differentiated verrucous carcinoma Ib stage, according to FIGO classification, in every case. Following radical hystectomy, the post-operative course was uneventful. The mean follow-up time was 4.6 years. The follow-up HPV tests of the H-SIL patients were consistently negative as to cytological and colposcopical findings and no relapse was observed. At one-year follow-up the ASC-H patient who had shown postoperative histological features of koilocytosis associated with CIN2 and VAIN 3 had a persistence of high-risk HPV types with negative cytological and colposcopical findings. The HPV test was positive for two years and colposcopic and histological findings of VAIN degree 2 were revealed three years after surgery. Following vaginal laser CO₂ vaporization the subsequently cytological and colposcopic checks were negative as was molecular detection of HPV. Conclusion: Follow-up colposcopy, cytology and molecular detection of HPV is recommended in all verrucous carcinoma patients since carcinogenic HPV DNA detection could represent a valuable diagnostic tool as an adjunct to cytology, for monitoring HPV infections with transforming potential.

Verrucous carcinoma, cervical lymphoma, cervical small cell carcinoma, and sarcomas (1-3) are rare neoplasia of the uterine cervix. Verrucous carcinoma is a rare variant of well-differentiated squamous cell carcinoma that is often confused with the more common condyloma acuminatum or papillary type of squamous cell carcinoma and it has a tendency to local invasion, without nodal or distant metastases.

Only 44 cases of cervical verrucous carcinoma have been described in the world literature, the other typical localizations are the vulva and vagina in the female genital tract, the male penis and the oral cavity. It is a primarily a tumour of the postmenopausal age and the involvement of the vulva and the cervix seems to be more common than involvement of the vagina. The patients with involvement of the uterine cervix seem to be slightly younger than patients with the tumour located in the vagina or in the vulva (4).

Infection with high-risk human papillomavirus (HPV) is the main cause of cervical intraepithelial and invasive neoplasias. HPV DNA has been detected in >90% of cervical carcinomas, with the most common HPV types identified as HPV 16, 18, 31, 33 and 45. Patients with persistent infection with these HPV types have a clearly enhanced risk of developing cervical carcinoma (5).

The aetiology of verrucous carcinoma of the female genital tract is still unknown although it has been associated with irritation and inflammation. Much evidence is accumulating indicating HPV as either the causative agent or a likely cofactor in the development of this lesion and it has been detected in approximately 25% of cases (6, 7).

The objective of this study was to evaluate the presence of HPV in verrucous carcinomas of the cervix and to

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investigate the persistence of high-risk HPV types as a predictor for the development of intraepithelial or invasive lesions of the lower genital tract, as an adjunct to cytology during the follow-up after treatment.

**Patients and Methods**

From 2001 to 2003, three patients, with atypical cytologies, attended to the Department of Gynecology, Perinatology and Child Health, University of Rome “Sapienza”. A personal history (age, parity, sexual habits, contraception, cigarette smoking, drug and alcohol consumption,) and written consent were available for every patient. All the patients underwent cytology and colposcopy followed by direct biopsy from any area revealing the greatest degree of abnormality. Preoperative cervical cytology specimens were collected in all cases from the ectocervix and from a cytobrush endocervical device. Cytological diagnosis was formulated in agreement with the Bethesda System (8). Colposcopy was performed by means of a Zeiss 50 T colposcope (Carl Zeiss Inc. Oberkochen, Germany), using 5% acetic acid and followed by Lugol test. Colposcopic findings were classified according to the International Classification (9). The Hybrid Capture II microplate method was used for molecular detection of low (6, 11, 42, 43, 44) and high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) HPV types as described by the manufacturer (Digene, Gaithersburg, MD, USA).

Trans-vaginal ultrasound tomography and pelvic contrast-enhanced magnetic resonance imaging (MRI) were performed to determine the regional extent of the tumour and for mapping nodal disease. A chest X-ray was performed preoperatively to exclude metastatic disease.

All the women with a histological diagnosis of verrucous carcinoma underwent a type II Piver radical hysterectomy, with bilateral salpingoophorectomy, upper vaginal third colpectomy and pelvic lymphadenectomy. Definitive histology and tumour staging according to the FIGO classification were obtained from surgical specimens (10).

In the first year after surgical treatment all patients were followed up at three-month intervals and subsequently six-month intervals for two years and then annually. Cytological Pap smear and colposcopy were carried out at the time of the follow-up checks, a direct biopsy was obtained from any suspicious area. The HPV tests were performed annually. Pelvic ultrasound tomography was performed every six months and a chest X-ray every year.

**Results**

The median age of the patients at diagnosis was 48.6 years (range 45-55). Two patients had more than 2 full term pregnancies and one patient was nulliparous. The median age at first intercourse was 16.6 years, the number of lifetime sexual partners was more than 2 for all three patients. In addition no patient declared condom use as a contraceptive method. No patients were alcohol users, one was a drug abuser and they were all cigarette smokers (more than five cigarettes daily).

Cytological findings revealed atypical squamous cells “cannot rule out high-grade lesion” (ASC-H) in one case and high grade squamous intraepithelial lesion (H-SIL) in two cases. In all the patients the Hybrid Capture test detected high risk HPV types.

Colposcopic findings showed cervical exophytic lesions measuring approximately 20 mm in two patients and 15 mm in the other one. The lesions appeared to be characterized by a white thickened epithelium, with an irregular cerebroid surface without defined borders, bloody, with atypical vessels and necrotic areas. The histology obtained by direct biopsy revealed a well differentiated verrucous carcinoma in every case.

The trans-vaginal ultrasound tomography and pelvic (MR) revealed neoformative processes with a diameter of 20 mm in two patients and 12 mm in the other one at the isthmo-cervical region. No pathological involvement of periterine or adnexal and parameters structures was described in any three patients. Moreover there was no evidence of adenopathy, no free endoperitoneal fluid and no distant disease at chest X-ray.

The post-operative course was uneventful and the three patients did not require any type of additional treatment. The post-operative tissues were examined macro- and microscopically and the histology confirmed the biopsy diagnosis of verrucous carcinoma.

Microscopic examination showed papillomatous tumours with a well-differentiated stratified squamous epithelium (Figure 1). The deeper layers were characterised by a pronounced hyperkeratosis and parakeratosis with rare mitotic figures.

There was no microscopic evidence of the spread of the malignant tumours outside the cervix and no metastases to the pelvic lymph nodes were observed. These histological findings were suggestive of Ib stage, according to the FIGO classification, in every case.

The patient with a preoperative cytological diagnosis of ASC-H revealed histological features of koilocytosis associated with cervical intraepithelial neoplasia grade 2 (CIN2) and vaginal intraepithelial neoplasia grade 3 (VAIN 3) coexisting with the verrucous carcinoma in the surgical specimen.

The patients regularly attended all the follow-up and no local recurrence or distant disease were reported. The mean follow-up time was 4.6 years (range 3-6 years).

In the women with a preoperative cytological diagnosis of H-SIL, the one-year follow-up HPV test was negative as to cytological and colposcopic findings. No relapse was evident in these cases during follow-up.

The patient with a preoperative cytological diagnosis of ASC-H, and postoperative histological features of koilocytosis associated with CIN2 and VAIN 3 had a persistence of high-risk HPV types with negative cytological and colposcopic findings at one-year follow-up. The remained HPV test positive for two years during the follow-
up checks and colposcopic and histological findings of VAIN degree 2 were revealed three years after Piver II surgery. This patient underwent vaginal laser CO2 vaporization. Subsequently the cytological and colposcopic and molecular detection of HPV gave negative results. At the last follow-up check all cases revealed negative HPV tests, cytological and colposcopical findings.

Discussion

The female genital tract is an uncommon location for verrucous carcinoma. Its histological characteristics were first described by Ackerman (11) as a slow-growing, locally aggressive tumour of the oral cavity. Because of the rarity of verrucous carcinoma, the histological diagnosis is usually difficult since it must be differentiated from condylomata acuminata, giant condyloma acuminatum (Buschke-Loewenstein tumour) and invasive squamous cell carcinoma (12, 13).

To distinguish this tumour from a benign condyloma, findings regarding the central connective tissue support are important. The fibrovascular cores of a verrucous carcinoma are limited to the superficial part of the tumour, in contrast to a benign condyloma, of which the fibrovascular cores extend from a deep layer to the surface (14).

An adequate biopsy of a verrucous carcinoma must show an equally well-differentiated bulbous rete-ridge pattern at the base of the lesion. These findings distinguish a verrucous carcinoma from a well-differentiated squamous carcinoma, that forms much keratin superficially but that might be relatively anaplastic and infiltrating in its deeper layers.

According to the report of Pantanovitz all three cases in this study showed a diagnostic cytology specimen although the analysis of earlier published data about the utility of cytological specimens obtained during colposcopy described a negative value of this procedure (15).

The HPV has been related (16, 17) to the development of squamous intraepithelial lesion of the cervix (SIL), carcinoma in situ and invasive squamous cell carcinoma, but the exact relation ship between verrucous carcinoma and HPV types is unknown. Several studies on HPV prevalence in verrucous carcinoma have been performed using different methods for the detection of HPV subtypes. Some case reports have shown HPV in verrucous carcinoma of the vulva and of the vagina, but several unsuccessful attempts to detect it in cervical verrucous carcinoma have been described in the literature (18-25).

In this report high-risk HPV types were detected in all three cervical invasive lesions. The association between HPV and this tumour could indicate that it is a causative or cofactor-like substance in the development of verrucous carcinoma.

Verrucous carcinoma has been described as a slow but relentlessly expanding tumour which in advanced stages or in recurrent lesions tends to be more aggressive (26). Radical surgery is the main factor improving the overall and disease free survival in these patients. Primary hysterectomy, with or without adjuvant radiotherapy, has achieved a 50% cure rate (27).

The multicentricity of pre-neoplastic intraepithelial lesions as CIN and/or VAIN associated with verrucous carcinoma, could be regarded as a surrogate marker for viral persistence and a prerequisite for recurrence. High-grade HPV or viral persistence has often been proposed as positive predictor value in identifying progressive and persistent lesions. A probable predictive value of HPV DNA for the monitoring of patients previously treated for verrucous carcinoma was observed in this study. Testing HPV DNA could be a useful technique in the triage of patients after surgery and it is necessary for determining whether cases affected by high risk HPV represent persistent or transient infections that will regress (28).

Follow-up colposcopy, cytology and molecular detection of HPV is recommended in all patients previously treated for malignant neoplasia. Even if cervical verrucous carcinoma is considered a rare variant of well-differentiated squamous cell carcinoma, the introduction of carcinogenic HPV DNA detection could represent a valuable diagnostic tool as an adjunct to cytology, for monitoring HPV infections with transforming potential.

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References

