Case Report
Non-HPV-related verrucous carcinoma of the endometrium: report of one case and review of literature

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Abstract: The clinicopathologic diagnosis and treatment of a case of endometrial verrucous carcinoma were analyzed, combined with the relevant literature for review and discussion. The patient was a 67-year-old postmenopausal woman who had recurrent vaginal bleeding with increased secretion. The gross morphology and histopathology of the uterine cavity tumor had typical characteristic changes of verrucous carcinoma. There was obvious inflammation in the tumor background and squamous differentiation in the residual endometrial gland basal reserve cells. Tumor cell immunohistochemistry showed wild-type P53, P16 negative expression and HPV molecular test was negative. The expression of p63 was positive in squamous differentiated reserve cells, and Ki67 showed high proliferative activity. Verrucous carcinoma of endometrium is a rare tumor with low malignancy, that belongs to a special subtype of squamous cell carcinoma. It is easily missed or misdiagnosed and has a long diagnosis cycle. The diagnosis should be combined with clinical data, imaging and pathology. The pathogenesis of the disease is not clear. Surgical treatment is the first treatment, and the clinical prognosis is good.

Keywords: Endometrium, verrucous carcinoma, reserve cells, non-HPV-related

Introduction
Verrucous carcinoma was first reported by Ackerman et al in 1948 [1]. It is a rare special subtype of well-differentiated squamous cell carcinoma with different clinical manifestations and histopathology from ordinary squamous cell carcinoma. The tumor grows slowly and the lesions are limited, mainly in the growing mode of exogenous verrucous and basal "push-extrusion", with good histologic differentiation. Early clinical manifestations and pathologic biopsy often lead to underdiagnosis or misdiagnosis due to lack of understanding. Current studies have shown that verrucous carcinoma in the female reproductive system is mainly found in vulva, vagina, cervix and other places [2-4], while verrucous carcinoma in the endometrium is extremely rare; so far only 5 cases have been reported [5-9]. The pathogenesis of the disease is not clear, so it is necessary to strengthen the understanding of the disease, improve the accuracy of early diagnosis, to avoid delay in treatment. This paper reports a case of verrucous carcinoma in the endometrium with immunohistochemical staining and molecular detection of human papillomavirus (HPV), and relevant literature has been reviewed.

Clinical data
Patient was a 67-year-old, Chinese female, menopausal for 20 years; She was well before, and had no history of estrogen use. In 2017, the patient developed slight vaginal bleeding with symptoms of increased secretion. In 2019, vaginal bleeding with increased secretion symptoms were aggravated, and the patient again went to the superior hospital for treatment. Results of ultrasonic examination: in the uterine cavity, there was a heterogeneous strongly echogenic mass, about 6.6 * 3.3 cm,
with unclear boundary, and dot-strip strong echo can be seen inside and the boundary while the myometrium is still clear. In addition, there are uterine fibroids with calcification. Pathology of uterine cavity curettage: benign squamous epithelial papilloma. Gynecological specialist examination: the cervix is smooth, no obvious abnormality is found, with retroposition of uterus, such as pregnancy of 2.5 months, with irregular shape, hard quality and good activity.

Marital and reproductive history: married at the age of 24, birth history 2-0-1-2, natural menopause at the age of 47. 40 years ago, “early pregnancy” caused an abortion. She has two girls, both in good health. The patient has no tumor-related family history.

Clinical preliminary diagnosis: the nature of uterine cavity occupation remains to be investigated, uterine fibroid. The patient required further surgical treatment, hysterectomy with bilateral adnexectomy and regional lymph node dissection were performed.

Materials and methods

The fresh specimens of uterus and bilateral adnexa were frozen for quick pathologic examination. Three endometrial masses were frozen for quick frozen section and staining. The rest of the specimens were fixed with 4% neutral formaldehyde, paraffin-embedded, sectioned and stained with H&E. The two-step method of envision was used for immunohistochemical labeling. The antibodies: ER, PR, HER2, Ki67, p16, p53, CK7 primary and secondary antibodies were all purchased from Roche company. The detection of HPV gene was carried out by arms fluorescence quantitative PCR, and the kit was purchased from Guangzhou Ambiping Co., Ltd.

Results

Characteristics of gross specimens

Uterus and bilateral adnexa: the size of the uterus was 12 * 9 * 6 cm, the length of the cervical canal 3.5 cm, the external diameter 3 cm. The cervix was still smooth, and a small polyp was seen in the cervical canal, with a diameter of about 0.5 cm. No obvious abnormality was found elsewhere. The uterine cavity above isthmus was filled with long and thin gray papillary masses with a range of about 8 * 7.5 cm, which were clearly demarcated with the isthmus (Figure 1A, 1B). The cut mass was mainly located in the mucosa, with a clear boundary with the muscular layer, and the focal area seemed to invade the superficial muscular layer (Figure 1C). There was a myoma under the fundic mucosa, 4 * 4 * 3.5 cm in size, with obvious calcification in the center. Bilateral adnexa showed no abnormality.

Histopathological features

(1) Low power microscopy showed diffuse verrucous hyperplasia of the masses in the uterine cavity and pushing growth of the basal part.
Some endometrial tissue remained in the deep part of the mass, and the periphery of the mass in the focal area moved with the abnormally differentiated endometrial area (Figure 2A-C). (2) High power microscopy showed that there was a thick layer of parakeratosis on the surface of the tumor. The spinous layer cells proliferated significantly, and vacuolated cells were found inside. The cytoplasm of the differentiated squamous cells in the middle and upper part was rich, with intercellular bridges, without obvious atypia. In the middle and lower part of the squamous cells, there was atypia such as increased cytoplasmic ratio, visible nucleolus, and karyomitosis being easy to see in the basal part. The basal part of the tumor grew in drumstick-shaped pushing type. In the deep part of the basement of the focus, there were small tumors infiltrating with abnormal cell differentiation (Figure 2D-F). (3) There were focal hyperplasia of squamous epithelium and papilloma like hyperplasia on the surface of the residual endometrial tissue around the main tumor body, and they moved with the tumor (Figure 2G, 2H). (4) Squamous cell differentiation and mucous cell differentiation were found in some glandular basal reserve cells of endometrium, in which P63 expressed in squamous epithelial differentiated cells was located in the basal part and arranged in single or multiple layers. Ki-67 showed high proliferation activity and continued with the basal part of tumor (Figure 2I-K). (5) In the deep endometrial stroma of the tumor, a large number of mixed plasma cells and lymphocytes infiltrated, and a large number of neutrophils gathered in the glandular cavity of some endometrial glands (Figure 2G, 2H, 2L). (6) The results of immunohistochemistry showed that P63 protein was positive; P16, ER, PR, HER2, CK7 protein was negative; P53 protein in tumor basal and parabasal cells were positive to different degrees; Ki67 protein showed that tumor basal and parabasal cells were positive (Figure 2M, 2N). (7) HPV molecular test results were negative (Figure 2O). (8) No adenocarcinoma was found in the uterine cavity. No obvious abnormality was found in cervix and cervical canal mucosa, and there was no sign of virus infection in cervix. No obvious abnormality was found in bilateral adnexa.

Pathologic final diagnosis: non-HPV-related verrucous carcinoma of the endometrium. Uterine leiomyoma with calcification, cervical polyp, and chronic cervicitis. No metastasis was found in regional lymph nodes.

After the operation, the patient was discharged smoothly without any follow-up treatment. At the time of writing this article, the patient had a good prognosis.

Clinical features

Verrucous carcinoma of endometrium is a highly differentiated squamous cell carcinoma with very low incidence. The clinical characteristics of five cases of verrucous carcinoma of endometrium reported in the literature and one case in this paper are summarized as follows (Table 1). The tumor mainly occurred in postmenopausal elderly women. The age of onset was 61-77 years old. Four cases were white and two were Asian. The main clinical symptoms of the disease are vaginal bleeding with increased secretion. Multiple histopathologic biopsies are often misdiagnosed as benign squamous hyperplasia or benign squamous papillary tumor due to good cell differentiation. In the follow-up process, the patient often went to see a doctor again or more times because of the unremitting symptoms, and finally diagnosed after hysterectomy. The interval of this process is generally 1-4 years. Its related pathogenesis is not well described yet. Clinical follow-up showed good prognosis after operation.

Discussion

Primary squamous cell carcinoma of endometrium was first reported by Gebhard in 1892 [10], and was rarely seen in the clinic [11, 12]. At present, the diagnosis needs to meet at least the following four criteria: (1) No primary squamous cell carcinoma in the cervix. (2) No correlation between endometrial mass and cervical squamous epithelium. (3) No adenocarcinoma in endometrium. (4) There must be clear evidence of squamous cell differentiation, such as presence of intracellular bridges and/or keratin [13, 14]. The study found that the pathogenesis may be associated with squamous metaplasia or multidirectional differentiation of basal reserve stem cells [5, 15, 16]. Primary verrucous carcinoma of endometrium is a special type of highly differentiated squamous cell carcinoma, that was first reported by Ryder in 1982 [5], and there are only five cases. Due to the extremely rare cases, the related pathogen-
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ic factors are not clear, and the clinical and pathological understanding of the disease is insufficient. Notably, it has been reported that patients did not get timely and accurate diagnosis and treatment at the first visit.

In this case, the tumor samples were fully selected and meet the above four basic diagnostic requirements, combined with its unique growth mode and referring to the HPV molecular test results, thus can be finally diagnosed as non-HPV-related endometrial primary verrucous carcinoma. Histopathology clearly showed that the reserve cells in the basal part of endometrial gland differentiated into squamous epithelium, and were continuous with the basal part of tumor. It was concluded that this change might be a precondition for the occurrence of endometrial verrucous carcinoma, which was consistent with Ryder’s report. In addition, squamous epithelium proliferated in different degrees around the tumor, and migrated with the verrucous carcinoma area. It is suggested that the histogenesis of endometrial verrucous carcinoma may have the following evolution process: under the action of related pathogenic factors → the reserve cells in the basal part of endometrial gland differentiate into squamous epithelium → Squamous focal hyperplasia/papilloma-like hyperplasia → cell dysplasia → verrucous carcinoma.

The patients are postmenopausal elderly women, most of them with no history of estrogen

**Table 1.** Clinical features of six cases of endometrial verrucous carcinoma

<table>
<thead>
<tr>
<th>Reporting year</th>
<th>Age and race</th>
<th>Clinical symptom</th>
<th>Pap smear</th>
<th>Interval period</th>
<th>HPV</th>
<th>P53</th>
<th>P16</th>
<th>Prognosis and metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 y</td>
<td>61 (white)</td>
<td>Increased vaginal secretions</td>
<td>Positive</td>
<td>2 years</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>good (-)</td>
</tr>
<tr>
<td>1988 y</td>
<td>64 (white)</td>
<td>Vaginal bleeding and increased secretions</td>
<td>Negative</td>
<td>2 years</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>good (-)</td>
</tr>
<tr>
<td>2000 y</td>
<td>77 (Asian)</td>
<td>Increased vaginal secretions</td>
<td>/</td>
<td>2 years</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>good (-)</td>
</tr>
<tr>
<td>2006 y</td>
<td>64 (white)</td>
<td>Vaginal bleeding</td>
<td>Negative</td>
<td>2 years</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>good (-)</td>
</tr>
<tr>
<td>2014 y</td>
<td>68 (white)</td>
<td>Increased vaginal secretions</td>
<td>Negative</td>
<td>2 years</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>good (-)</td>
</tr>
<tr>
<td>This case</td>
<td>67 (Asian)</td>
<td>Vaginal bleeding and increased secretions</td>
<td>NA</td>
<td>2 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>good (-)</td>
</tr>
</tbody>
</table>
use before treatment. The more severe endometritis in the background of tumor may be related to the lower level of estrogen in the elderly, and persistent inflammatory stimulation is an important factor that leads to squamous epithelial differentiation and tumor progression of endometrial gland reserve cells. There are case reports showing strong positive expression of P53 protein in endometrial verrucous carcinoma, suggesting the presence of P53 tumor suppressor mutation; however, in this case, the tumor showed that P53 protein was strongly or weakly positively expressed, which may be wild type, indicating that P53 gene mutation may be related to the pathogenesis of some cases. The newly reported cases and routine HPV molecular tests of this case showed negative results, suggesting that there was no clear correlation between the incidence of endometrial verrucous carcinoma and HPV infection. In addition, there may be racial differences in the incidence of endometrial verrucous carcinoma. At present, the incidence in white people is relatively high, while that in Asians is relatively low. To sum up, primary verrucous carcinoma of endometrium is extremely rare, and its pathogenesis is limited, and more case results are needed to provide reference. The verrucous carcinoma is dominated by local invasive growth. Rare occurrence of regional lymph nodes and distant metastases are the most prominent clinical features. At present, hysterectomy with bilateral adnexectomy is the main treatment. All the six cases showed good prognosis after operation, because the uterus was relatively free and the uterine cavity showed a relatively closed space. No follow-up treatment was performed after operation. However, there is a certain probability of recurrence and metastasis of this kind of tumor, so long-term clinical follow-up is necessary.

Endometrial verrucous carcinoma mainly needs to be distinguished from uterine cavity benign condyloma acuminatum and squamous cell carcinoma: (1) The vascular axis of benign condyloma acuminatum extends deep, while the vascular axis of verrucous carcinoma is limited to the surface. (2) Squamous cell carcinoma has a unique solitary tumor cell nest with an obvious invasive growth pattern, while verrucous carcinoma has a unique verrucous structure and a “swelling” growth pattern at the base.

Combined with the diagnosis and treatment of this case, the literature was reviewed and summarized. Verrucous carcinoma of the endometrium gives us the following warnings: (1) Site specificity: there is a certain interference in the cervical site; verrucous carcinoma is more common in the cervix than endometrium. The diagnosis of verrucous carcinoma of the endometrium must be excluded from the possibility of cervical lesions, which is more difficult to diagnose than other parts. We suggest that the site of the lesion needs to be identified in combination with clinical and related examinations. (2) It is difficult to diagnose by curettage and biopsy: clinical curettage and biopsy are often superficial. Cytological morphology is not typical, which makes pathologic diagnosis difficult. Thus the clinical biopsy materials should be wide and deep, and should be taken to the base of the tumor as far as possible, and the specimens should be repeat sampled if necessary. (3) Insufficient clinical and pathological understanding: the incidence of this disease is too low, and biopsy pathology is often misdiagnosed as benign squamous epithelial lesions due to good cell differentiation. Clinical doctors lack understanding of the disease, so it is easy to neglect and delay treatment. In summary, when multiple curettage and biopsy of uterine cavity show squamous epithelial hyperplasia with obvious keratosis, the clinician and pathologist should be alert to the possibility of the disease, communicate in time, and make comprehensive diagnosis and treatment in combination with relevant examination.

Disclosure of conflict of interest

None.

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