Int J Clin Exp Pathol 2019;12(9):3426-3432
www.ijcep.com /ISSN:1936-2625/IJCEP0098305

Original Article

Primary extramammary Paget's disease: a clinicopathological study of 28 cases

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Received June 11, 2019; Accepted July 22, 2019; Epub September 1, 2019; Published September 15, 2019

Abstract: To analyze the clinical and histopathological manifestations, immunohistochemistry, treatment, and prognostic factors of primary, extramammary Paget's disease (EMPD), we systematically reviewed the clinical presentations, histopathology and follow-up courses of 28 patients with primary EMPD. Clinically, their symptoms and morphology mimicked various types of dermatoses, such as seborrheic dermatitis, eczema, candidiasis, tinea cruris and erythrasma, so the initial diagnosis of EMPD was often delayed or missed. Histopathology showed invasive EMPD, and the tumor cells were mostly solid nests or had a glandular structure. The cellular atypia was obvious and signet ring Paget's cells could usually be observed. The acantholysis phenomenon in the epidermis could be seen. The condition was associated with stromal invasion, lymphatic metastasis, and even vascular invasion. Adnexal involvement in primary EMPD was a very common feature. The immunohistochemical markers CK7, GCDFP-15, CEA and HER-2 positive can identify other tumors similar to Paget's disease. We concluded that invasive EMPD is a rare malignant skin neoplasm with morphological diversity. Poorly differentiated cell morphology, extensive adnexal involvement, and an invasive pattern of solid sheets are significantly associated with lymph node metastasis and a worse prognosis. Pathologists should be alert to invasive lesions and make the correct diagnosis.

Keywords: Extramammary Paget's disease, pattern of invasion, diagnosis, immunohistochemistry

Introduction

Extramammary Paget's disease (EMPD) is a rare malignant disorder of skin that occurs on apocrine sweat gland-bearing skin with abundant hair follicles [1]. The most common site is the vulva, followed by perineal, perianal, scrotal and penile skin. Other rarer sites are the axilla, the buttocks, the thighs, the eyelids, and the external auditory canal [2]. EMPD usually manifests as erythematous or persistent, eczema-like skin lesions [3]. EMPD has also been called skin in situ adenocarcinoma. The tumor is confined to the epidermis, it grows slowly, and distant metastasis is rare. However, cases of dermal infiltration and lymph node metastasis of EMPD have been reported in recent years [4, 5]. We statistically analyzed 28 cases of primary EMPD admitted to our hospital from May 2006 to May 2014, including 24 cases of in situ lesions and 4 cases of invasive lesions. We performed histological observations on 28 cases of EMPD and studied, using immunohistochemical methods, 4 cases of invasive EMPD. We focused on their diagnosis and differential diagnosis in order to raise our awareness of such lesions and to explore the prognostic factors for this rare condition.

Materials and methods

We collected 28 cases of primary EMPD at the First Affiliated Hospital of Bengbu Medical College from May 2006 to May 2014, including 12 cases that occurred in the scrotum, 6 cases in the vulva, 4 cases in the perianal area, 3 cases in the armpit, 2 cases in the penis, and 1 case in the buttocks. There were 23 males and 5 females, and there were 24 in situ lesions and 4 cases of infiltration and metastasis. The HE-stained sections (4 um thickness) were reexamined to evaluate the tumors' histological features, and immunohistochemistry was performed using the Elivision technique. The antibody details are given in **Table 1**. The clinical demographics and follow-up data were obtained

Table 1. Sources of the antibodies used in the immunohistochemical analysis

Source	Antibody
CK	Monoclonal, clone AE1/AE3
CK7	Monoclonal, clone OV-TL 12/30
CK20	Monoclonal, clone Ks20.8
CK5/6	Monoclonal, clone D5/16B4
GCDFP-15	Monoclonal, clone 23A3
CEA	Monoclonal, clone ZC23
HER-2	Monoclonal, clone CB11
Ki-67	Monoclonal, clone MIB-1
CDX-2	Monoclonal, clone AMT28
p63	Monoclonal, clone 4A4
Melanoma	Monoclonal, clone HMB45

All antibodies were obtained from Maixin Biotech, Inc. (Fuzhou, China), and were ready to use.

from medical records and the referring physicians. All antibodies were obtained from Maixin Biotech, Inc. (Fuzhou, China), and were ready to use. This study was approved by the Ethics Committees of the First Affiliated Hospital of Bengbu Medical College and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Results

Clinical features

The ages at the time of the patients' diagnoses ranged from 45 to 79 years, with an average of 64 years. Clinically, the skin lesions initially presented as erythema or as an eczematous eruption, then they progressed to scaly or crusted infiltrated plaques, sometimes with erosion or ulceration. The lesions were present for 4 months to 15 years before surgical removal (average duration, 49 months). The symptoms were not relieved after applying antiviral and fungal drugs by themselves. In the cohort, 25 complained of pruritus or tenderness. Nodules or masses were palpable in 3 cases, varying from 0.5 cm to a maximum dimension of 1.5 cm. 4 cases had multifocal lesions with positive margins. 3 cases had lymph node metastasis. 19 cases were focal and 9 cases were extensive. In the largest lesions, a large, well-defined erythematous plaque was observed extending from the suprapubic area and involving the skin covering almost the whole of the scrotum and the shaft of the penis.

Pathological examination

The general examination of 28 specimens showed a thickening of the skin, erythema or grayish white in color, partial surface erosions, and irregular boundaries. A microscopic examination revealed that the tumor cells of the in situ EMPD were only found in the epidermal layer and were either scattered or nested (**Figure 1A**). The Paget cells were mainly located at stratum basale or the lower part of stratum spinosum. The tumor cells were large and round, and the cytoplasms were lightly stained. The nuclei were round or slightly irregular, centered, and medium-sized, and mitosis was rare. The lesion could be multifocal. For invasive EMPD, the Paget cells were larger, the nucleoplasmic ratio was significantly increased, the nuclei were irregularly deeply stained, and the mitosis was easy to see. Paget cells could even be of a signet ring shape, with clear cytoplasms and mucus. Tumor cells often occupied the entire epidermis (**Figure 1B**). The tumors were mostly nested, of various sizes, and irregular in shape. Glandular formation with true lumina within the epidermis was found in 2 cases, and signet ring cells were seen in 1 case.

The acantholysis phenomenon in the epidermis can be seen (**Figure 1C**). The involvement of the cutaneous adnexa including the sweat glands and folliculi pili was observed in the invasive EMPD cases (**Figure 1D**). Of the 4 invasive lesions, 1 case occurred in the vulva, and Paget cells with large nuclei and prominent nucleoli were scattered singly or in clusters throughout the entire epidermis and broke through the basement membrane. We can see small or medium-sized nests under the epidermis and can even find lymph node metastasis or intravascular tumor thrombus (**Figure 2A-D**). In the 2 scrotum cases, a large number of invasive tumor cells were arranged in a nested form or solid sheets can be seen under the epidermis, and the bilateral inguinal lymph nodes were widely metastasized. One case that occurred in the perianal area showed a large number of signet ring cells in the whole epidermis, the intracellular mucus was abundant, and the infiltrated area showed an mucinous adenocarcinoma image (**Figure 3A**).

Immunohistochemical features

Immunohistochemical staining was performed in 19 patients in this group. The positive rate of

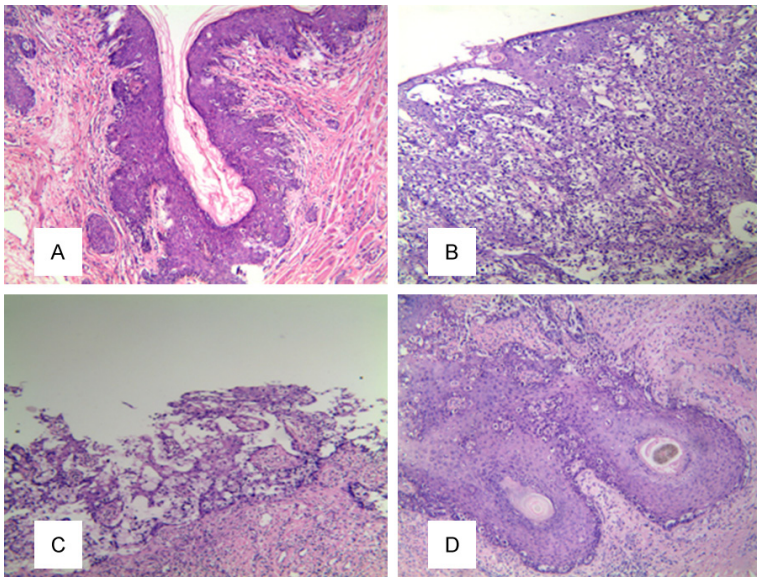

Figure 1. A. Paget cells can be seen individually scattered or nested within the epidermal layer. B. Paget cells of invasive EMPD occupied the entire epidermis. C. The acantholysis phenomenon in the epidermis can be seen. D. Involvement of cutaneous adnexa, including the sweat glands and follicluli pili, was observed.

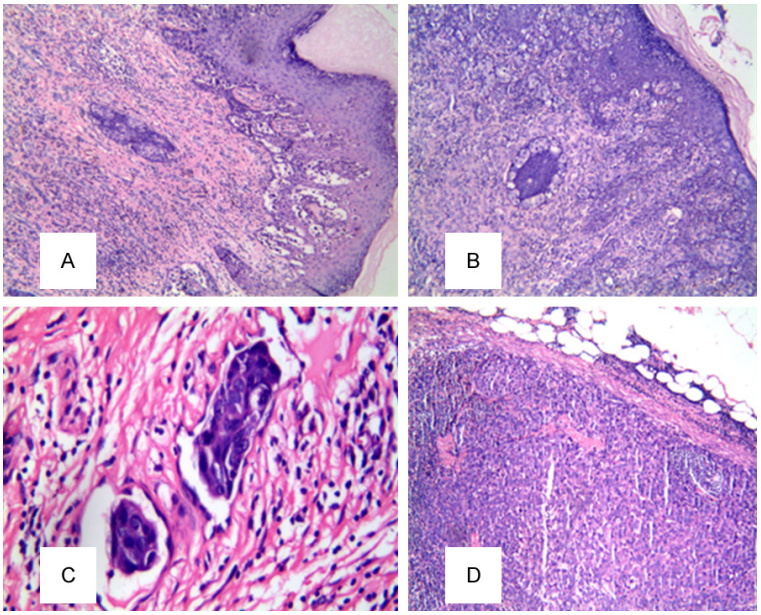

Figure 2. A. Paget cells were scattered singly or in clusters and broke through the basement membrane. B. We can see small or medium-sized nests under the epidermis. C. We can see intravascular tumor thrombus. D. We can see lymph node metastasis in invasive EMPD.

CK7 in the tumor cells was 100% (19/19) (**Figure 3B**). CEA was positive in most tumor cells, and the positive rate was 84.2% (16/19), The HER-2 positive rate was 57.9% (11/19), and the GCDFP-15 positive rate was 52.6% (10/19). The positive rate of AB was 47.4% (9/19). The tumor cells were negative for CDX2, CK20, CK-5/6, P63, CD3, CD43, HMB45, and therefore excluded the possibility of the urinary digestive tract, Bowen's disease, and melanoma.

Follow-up

During a median follow-up period of 46.5 months (range: from 6 months to 5 years), we observed recurrence in 5 patients. The time of recurrence was 6 months to 2.5 years after surgery, and the recurrence rate was 17.8% and they underwent a repeated surgery. All the patients were in good condition after the follow-up.

Discussion

Paget's disease, also known as skin eczema-like cancer is usually divided into mammary and extramammary types [6]. EMPD often occurs in older patients on areas rich in apocrine sweat glands such as the vulva, scrotum, perineum, perianal area, and axilla. The clinical features can be characterized by skin erythema, eczema or perianal disease, such as anal pruritus, anal fistula, etc. [7]. EMPD is often misdiagnosed because of atypical symptoms. When the diagnosis is confirmed, the lesion usually has dermal infiltration and lymph node metastasis, which eventually leads to death. For the above-mentioned parts of the body with long-term skin eczema-like lesions, close attention needs to be paid to them, and if necessary, a biopsy may be needed.

In the diagnosis of primary Paget disease, we must carefully exclude secondary lesions. EMPD is often associated with synchronous or metachronous malignancies in the underlying dermis and adjacent or distant organs [8]. The most frequently associated carcinomas are

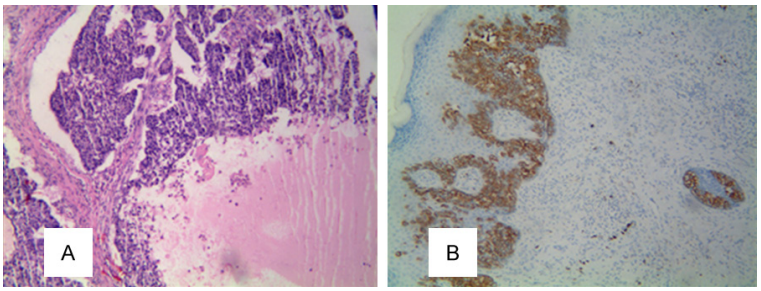

Figure 3. A. The infiltrated area shows a mucinous adenocarcinoma image in one invasive EMPD case. B. Immunohistochemical staining shows that CK7 was strongly positive in the Paget cells.

sweat gland adenocarcinoma in the underlying dermis or colorectal carcinoma, prostate carcinoma, and urothelial carcinoma in the adjacent organs. Under these circumstances, immunohistochemical markers are helpful [9]. Paget-like dissemination of rectal and anal ductal carcinoma is positive for CK20 and negative for CK7 and GCDFP-15. The dissemination of urothelial carcinoma is often positive for CK7, CK20 and negative for GCDFP-15 and CEA. In the intraepithelial dissemination of cervical adenocarcinoma, the positive rate of HPV is higher. Primary Paget's disease often expresses CK7, CEA, GCDFP-15, and HER-2, and mucus staining is also positive. Notably, CK7 is strongly positive in Paget cells. Single scattered or micro-invasive lesions under the epidermis or even the Paget cells at the edge of the lesion in the epidermis could also be marked by CK7 immunohistochemical staining. It has been reported in the literature [10] that CK7 can be used to label the tumor margin and microinvasion under the epidermis, which is a good indicator for the difficulty of determining whether there is tumor cell residue at the edge of the tissue in the conventional sections.

In one case of perianal Paget's disease in our group, mucinous adenocarcinoma was found under the epidermis. After a detailed examination, no deep tissue cancer found, and the immunohistochemistry was positive for CK7, CEA, and AB/PAS. Because CK20 and GCDFP-15 were negative, this also support the conclusion that this was primary Paget's disease of the perianal skin rather than other tumor tissue invading the epidermis. There was no recurrence or metastasis after 6 months of follow-up. In the traditional concept, Paget's disease is a kind of skin in situ adenocarcinoma, and most patients have good prognosis because EMPD progresses slowly and is usually limited to the epidermis and the cutaneous adnexal structures [11].

However, another female patient we encountered with vulvar Paget's disease had received five-year's treatment for vulvar skin disease. When she was diagnosed, the lesion not only had dermal infiltration but also had vascular invasion and extensive lymph node metastasis. This case had no obvious solid tumor under the epidermis but an invasive growth of tumor cells singly or in clusters could be found. It is worth mentioning that the tumor cells extensively involve hair follicles and small sweat glands under the dermis. The other 2 cases occurred in scrotum, and Paget cells proliferate in nodular or medium-sized nests in the epidermis and invade the dermis.

Comparing these 4 cases of invasive Paget's disease and other in situ lesions, we find that Paget cells of invasive EMPD are more widely distributed in epidermis, and some areas even involve the entire epidermis. The cells are larger, the nucleoplasmic ratio is significantly increased, and nuclear division is easy to see. Sometimes signet ring cells can be seen, and their cytoplasms are translucent and contain mucus. The tumors are mostly solid nested or glandular tubular structures. The acantholysis phenomenon in the epidermis can also be seen. Adnexal involvement is a common finding in large samples as well as in small biopsy specimens [12]. In limited reports on invasive EMPD, some authors have suggested that the high nucleoplasmic ratio, the form of the signet ring shape, and the invasive pattern of solid sheets were a poorly differentiated manifestation and were significantly associated with both lymph node metastasis and a worse prognosis [13]. This agrees with our observation.

Primary EMPD is a form of intraepithelial adenocarcinoma of uncertain histogenesis, for which cutaneous adnexa, clear cells of Toker, pluripotent stem cells, and anogenital mammary-like glands (AGMLG) have been proposed as possible sites of origin [14]. The theory of abnormal differentiation of Toker cells and pluripotent stem cells provides the theoretical

roots of infiltration and metastasis of the original Paget's disease and also explains the common "jumping" phenomenon between tumors in the epidermis [15].

According to the literature, EMPD with dermal infiltration accounts for about 15-20% of the original Paget's disease, but if the material is insufficient, infiltration may not be found [16]. Some scholars [17] observed that tumor cells in the microinvasive EMPD were scattered foci of superficial dermal invasion similar to the early infiltration of squamous cell carcinoma. The tumor cells broke through the basement membrane, so the depth of infiltration below the normal squamous epithelium ≤ 1 mm was defined as microinvasive Paget's disease, which did not affect the overall prognosis. Shiomi et al. [18] found that lymph node metastasis was significantly more frequent in EMPD cases with dermal invasion more than 1 mm. Reviewing the three cases with lymph node metastasis in our cohort, the multiple infiltrates are > 1 mm. Even more striking is the fact that Paget cells in metastatic cases are extensively involved in skin appendages. Nomura et al. [19] have observed that Paget cells can directly invade the dermis without passing through the basement membrane and expand down through the skin appendages. Konstantinova [20] observed 178 cases of primary EMPD lesions and found that Adnexal involvement in primary EMPD is a very common feature and serves as a pathway for carcinoma to spread into deeper tissue. Hair follicles and eccrine ducts are the most commonly affected adnexa by Paget cells. This phenomenon should be taken into account when planning topical therapy or developing novel local treatment modalities for EMPD. This was also a common feature in our group. At the same time, different degrees of the acantholysis phenomenon can be seen in the epidermis, which indicates that the adhesion of the tumor cells has decreased or is lost. The presence of the acantholysis phenomenon in primary Paget's disease may also be a high risk factor indicating tumor recurrence and metastasis [21].

To explore the reasons why EMPD is easily misdiagnosed and delayed, we find the clinical features of this lesion confusing. The differential diagnosis should consist of two parts. From the clinical perspective: it includes eczema, seborrheic dermatitis, perianal disease, hemorrhoids, anal fistula, etc. In elderly patients with eczema-like plaque with refractory pruritus, the application of corticosteroids cannot be alleviated, so EMPD should be highly suspected. Perianal ulcers or long-term unhealed anal fistulas, after excluding other diseases, should also consider EMPD as a diagnosis. At this time, a pathological biopsy is needed. From the perspective of histopathology, the differential diagnosis includes Bowen's disease, malignant melanoma, and lymphoma. In addition to the clinical and histological features, an immunophenotype can also be helpful. Bowen's disease originates from squamous cells and often expresses p63 and high molecular weight keratin, but it does not express CK7, CEA, or GCDFP-15, and mucus staining is negative. Tumor cells of malignant melanoma express HMB-45 and S-100, but do not express CK7. Skin lymphoma invasion of the epidermis is usually T cell lymphoma, expressing LCA, CD3, and CD43 but not CK7, CEA, or GCDFP-15 and so on [22].

In terms of treatment, surgical resection is currently the main treatment method, but the key to achieving good results is early diagnosis and early surgical treatment, and surgical resection should ensure sufficient range and depth. All patients should be followed up for a long time after surgery. After the operation, if there is a similar eczema-like change in the surgical site or in other parts, a biopsy should be performed in time to exclude recurrence.

It can be seen that for EMPD, both clinicians and pathologists should pay more attention to it. If such lesions are suspected, a pathological biopsy should be taken promptly to make a clear diagnosis. Pathologists should pay more attention to the morphological characteristics of Paget cells. After a differential diagnosis and the exclusion of similar lesions, the possibility of infiltration and metastasis should be considered for the poorly differentiated cell morphology or the extensive adnexal involvement. Therefore we should correctly determine the stage of the disease, give appropriate treatment, and reduce the risk of recurrence and metastasis.

Acknowledgements

We would like to thank to Editage for help with English language editing. Thanks also to Dichen Li and Qun Xie (Department of Pathology, the

Frist Affiliated Hospital of Bengbu Medical College) for their assistance with the histopathological and immunohistochemical stain evaluations. This study was supported by the Natural Science Foundation of Anhui Province (no. 1608085-QH207), the Key Supporting Project of Prominent Youth in Universities of Anhui (no. gxyqZD2016161), the Natural Science Foundation of Bengbu Medical College (nos. BYKF1707, BYKF1711, and BYKF1712), and the University Student Innovation and Entrepreneurship Program of Anhui Province (no. 201810367084).

Disclosure of conflict of interest

None.

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