Jpn. J. Cancer Res. 80, 19–23, January 1989

Detection of Human Papillomavirus Type 16 DNA and Papillomavirus Genus-specific Antigens in Vulva and Cervix from Patients with Bowenoid Papulosis

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The warty disordered lesions of the vulva in three female patients were diagnosed as Bowenoid papulosis on the basis of clinical and histopathological findings. In all three vulvar lesions, human papillomavirus type 16 (HPV 16) DNA was identified by Southern blot hybridization and papillomavirus genus-specific (PGS) antigen was detected in one case immunohistochemically. Furthermore, colposcopic examination revealed the presence of abnormal uterine cervical lesions in two cases. They were found to be intraepithelial neoplasia which harbored HPV 16 DNA and were positive for PGS antigen.

Key words: Bowenoid papulosis — HPV 16 — Female genital tract

Bowenoid papulosis is a unique warty disease of the anogenital region and its histological features are difficult to distinguish from those of carcinoma *in situ* of the skin, Bowen's disease. The clinical course of Bowenoid papulosis is generally benign.[1)] In 1983, Ikenberg *et al.* reported for the first time that human papillomavirus type 16 (HPV 16) DNA was detected in 8 out of 10 biopsies from Bowenoid papulosis of male and female patients.[2)] HPV 16 has been most frequently found in uterine cervical cancer.[3–5)] In addition, Obalek *et al.* reported that the cervical lesions of some female patients with Bowenoid papulosis were intraepithelial neoplasia (CIN) which were positive for HPV DNAs but not HPV 16.[6)] There has, however, been no report on association of HPV with Bowenoid papulosis of Japanese patients. Considering that HPV might be an etiological agent of genital tumor, it is important to know what type of HPV induces what type of clinical and pathological changes. In this report, we examined the vulva, vagina and cervix of three Japanese patients with Bowenoid papulosis clinically and histopathologically and we tried to identify the types of HPV present in these lesions by Southern blot hybridization.

MATERIALS AND METHODS

We examined three female patients who have attended our out-patient clinic during the last 2 years. Their ages were 50, 31 and 20 years, respectively. In all patients, biopsy of vulvar lesions was performed several times while cervical lesions were also taken under colposcopy. Biopsy specimens were divided into two parts. One part was fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections (3 μm thickness) were stained with hematoxylin and eosin (H-E) for histopathological examination and were subjected to the peroxidase-antiperoxidase (PAP) test with rabbit antiserum against papillomavirus genus-specific (PGS) antigen (diluted to 1:5000, Dako Corp., Santa Barbara, Calif.).[7)] Counterstaining was performed with Giemsa solution. The other part was digested with 100 μg/ml proteinase K in 50 m*M* Tris-HCl (pH 8)-2% sodium dodecyl sulfate-50 m*M* EDTA at 37°C overnight, then treated with 100 μg/ml RNase at 37°C for 2 h. Cellular DNA was collected by precipitation with ethanol after three extractions with phenol/chloroform/isoamyl alcohol (25:24:1). Southern blot hybridization was done as follows; 20 μg of total cell DNA was digested with *Pst* I enzyme and was electrophoresed in a 1% agarose gel and then separated DNA was transferred to a nitrocellulose filter by the method of Southern.[8)] Hybridization was performed at 42°C under either nonstringent (20% formamide, 5× SSC) or stringent (50% formamide, 5× SSC) conditions using ^{32}P-labeled HPV 16 and/or HPV 6 DNAs. The filters were washed with 2× SSC, 0.1% SDS at 48°C (nonstringent) or with 0.2× SSC, 0.1% SDS at 60°C (stringent).

RESULTS

Clinical and histopathological findings The vulvar lesions of the three patients were multifocal, brown-colored and partially confluent papules on the labium majus and labium minus. Their histological features were atypical epidermal hyperplasia with hyperkeratosis or parakeratosis, resembling Bowen's disease (Fig. 1). The epidermal cells partially showed moderate to severe atypia with various degrees of pleomorphism and hyperchromatic irregular nuclei, being less atypical in other portions with nuclear uniformity. The presence of

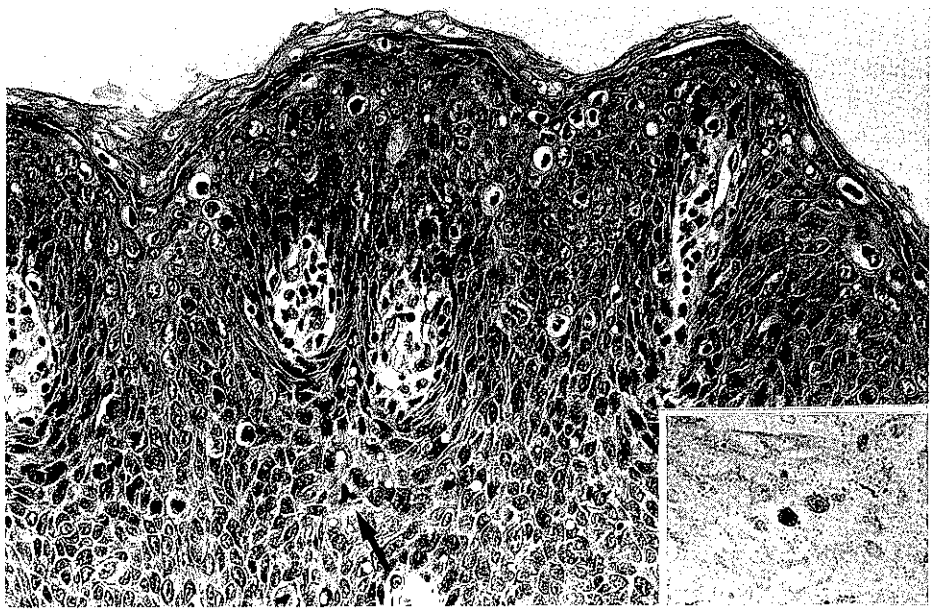


Fig. 1. Section of Bowenoid papulosis (Case 2) shows epidermal hyperplasia with cellular atypia and pleomorphism, resembling Bowen's disease. Koilocytes and parakeratocytes are shown in the upper part of the epidermis. Numerous mitotic figures, in part abnormal (arrow), are characteristic. H-E stain, ×250. Inset: Immunoperoxidase staining. PGS antigen is localized in the nuclei of koilocytes. PAP method, ×250.

Table I. Bowenoid Papulosis in Women

Patient No.	Age (yr)	Lesion	Histology	PGS antigen	HPV 16 DNA
1	50	vulva	Bowen's atypia	−	+
		cervix	chronic cervicitis	−	−
2	31	vulva	Bowen's atypia	+	+
		cervix	mild dysplasia	+	+
3	20	vulva	Bowen's atypia	−	+
		cervix	mild dysplasia	+	+

dyskeratotic cells, multinucleated giant cells and numerous mitotic figures (partly abnormal) was characteristic. Although rete ridges were elongated and thickened with papillomatosis, the basement membranes were intact. In Case 2 only, perinuclear vacuolization and koilocytotic changes were observed focally in the upper layer of the epidermis, the nuclei of which were positive for PGS antigen immunohistochemically (Fig. 1, inset). Since both clinical and histopathological findings of our cases were identical to those of the previous report,[1] we diagnosed them as Bowenoid papulosis (Table I).

On the other hand, colposcopic examination of the vaginal wall and the uterine cervix of these patients revealed abnormal findings of the cervix in two patients (Cases 2 and 3), displaying acetowhite lesions. Their histological features included CIN showing mild dysplasia with koilocytosis, and some koilocytes were positive for PGS antigen (Fig. 2 and inset). Case 1 showed normal appearance and was diagnosed as chronic cervicitis histologically (Table I). There was no colposcopic abnormality on the vaginal wall of these three patients.

HPV 16 DNA in vulvar and cervical lesions For screening HPV DNA in these lesions, blot hybridization was done at first under nonstringent conditions with ^{32}P-labeled HPV 6 and 16 DNA probes. Only the characteristic *Pst* I digested fragments of HPV 16 DNA were detected in these specimens, indicating the absence of any

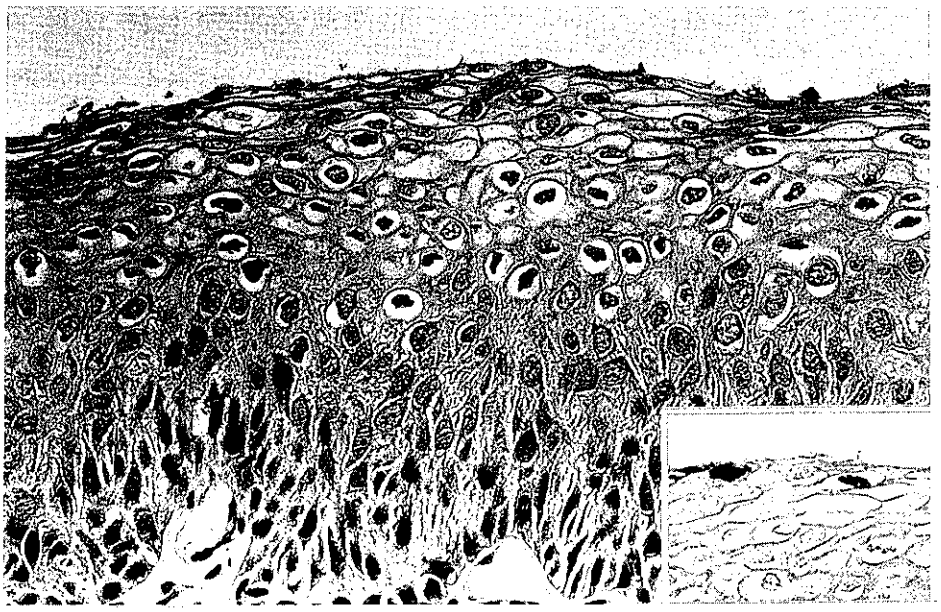
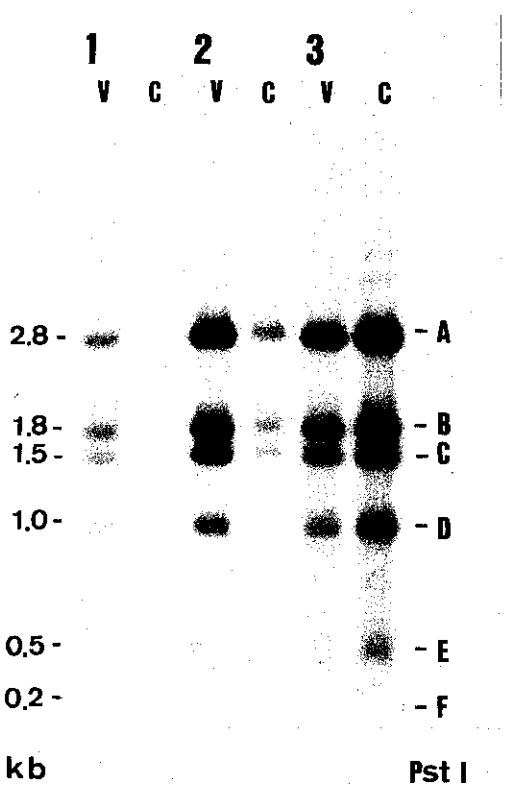


Fig. 2. Section of the acetowhite lesion of the cervix (Case 3) shows CIN featuring mild dysplasia with koilocytosis. H-E stain. ×400. Inset: Immunoperoxidase staining. PGS antigen is localized in the nuclei of the most superficial cells of the epithelium with koilocytosis. PAP method. ×400.



other types in these lesions (data not shown). This result was confirmed by an experiment under stringent conditions with HPV 16 probe. As shown in Fig. 3, all three vulvar lesions (V) harbored HPV 16 DNA, presenting six fragments of authentic HPV 16 (A to F on the right margin), although longer exposure of the blot was necessary for detection of the *Pst* I-F fragment. Furthermore, in two CIN lesions (C, Cases 2 and 3) HPV 16 DNA was also detected, but no HPV DNA was found in the cervix of Case 1. The nature of two off-sized bands (seen in C, Case 3) was not clear, but the physical states of all these viral molecules were found to be episomal (data not shown).

DISCUSSION

Clinical features that differentiate Bowenoid papulosis from Bowen's disease of the genitalia include 1) onset at

Fig. 3. Southern blot hybridization analysis of HPV 16 DNA in the vulvar and cervical lesions of three female patients with Bowenoid papulosis. Total cell DNA (20 μg) was digested with *Pst* I and electrophoresed in a 1% agarose gel. The filter was hybridized to ^{32}P-labeled HPV 16 DNA under stringent conditions. The characteristic *Pst* I pattern of HPV 16 (A to F on the right margin) was detected in all vulvar lesions (V) and in two cervical lesions (C). The molecular weight of the six fragments is indicated in kilobases (kb) on the left margin.

an earlier age on average, 2) appearance of multicentric pigmented papules, 3) frequent occurrence of spontaneous regression, and 4) simultaneous or previous complication with condyloma acuminata, though it is still difficult to distinguish it from Bowen's disease histologically.[1, 6, 9–11] Diagnosis of our cases was based upon both clinical and histological findings.

The preferential association of HPV 16 DNA in female Bowenoid papulosis was further confirmed by our result. In the previous reports, HPV 16 has been detected in 3 positive cases out of 3 patients (3/3)(Germany),[2] 2/2 (Switzerland),[12] 2/2 (USA),[13] and 4/5 (Poland)[6] without geographical differences. The frequency of the association with HPV 16 DNA in Bowenoid papulosis is much higher than that of HPV 16 in cervical cancer.[3–5] The physical state of viral DNA in the lesion was found to be episomal, in agreement with the previous report.[14] However, Bergeron *et al.* found that the viral DNA was integrated within cell DNA.[13]

In the cervix, we found CIN in 2 out of 3 patients. Similarly, Obalek *et al.*[6] reported CIN in 3 out of 5 patients and Rüdlinger[12] noted carcinoma *in situ* in 4 out of 10 patients. These findings suggested that female patients with Bowenoid papulosis frequently have some pathological changes in the cervix. Obalek *et al.* identified types of HPV in two CIN cases, i.e., HPV 16 related DNA in one case and HPV 33 DNA in the other case, being different in type from HPV 16 present in Bowenoid papulosis. We detected the same HPV 16 in CIN lesions of two cases and in the matched vulvar lesions simultaneously. Considering that HPV 16 has been most frequently found in cervical cancer, the female patients with Bowenoid papulosis might be at high risk for cervical cancer.

Immunohistochemical examination showed the presence of capsid or virion antigen of HPV. Some vulvar specimens from Bowenoid papulosis have been positive for PGS antigen.[1, 6, 15–18] We also detected the PGS antigen in one vulvar lesion and in two cervical lesions. It may be that these antigen-positive lesions would act as a reservoir for viral spread.[1] There was no correlation between the amount of HPV 16 DNA and that of PGS antigen and it seems likely that the synthesis of virus particle or viral capsid antigen is not effective in these tissues.[19]

Both vulvar and cervical lesions of these patients disappeared after repeated partial excisions (vulva) and multiple punch biopsies (cervix) and recurrence has not been observed for two years since the visits to our clinic. Careful long-term follow-up study is important to evaluate the real prognosis of Bowenoid papulosis together with CIN in these cases. Finally, we wish to emphasize that colposcopic examination of the cervix and the vaginal wall should be performed for patients with Bowenoid papulosis and also for the sexual partners of male patients with Bowenoid papulosis.

ACKNOWLEDGMENTS

We are grateful to Dr. H. zur Hausen for providing cloned HPV 6 and HPV 16 DNAs. We thank Dr. S. Koi for valuable suggestions and Dr. T. Kurata for a critical reading of the manuscript.

(Received August 17, 1988/Accepted November 22, 1988)

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