

SHORT COMMUNICATION

Human Papillomaviruses in Anogenital Epithelial Lesions

Madoka Kato¹, Akira Shimizu^{1*}, Yuko Takeuchi¹, Tomoyasu Hattori¹, Masatoshi Abe¹, Hiroo Amano¹, Sei-ichiro Motegi¹, Atsushi Tamura² and Osamu Ishikawa¹

Department of Dermatology, ¹Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, and ²Isezaki Municipal Hospital, Japan.

*E-mail: ashimizu@med.gunma-u.ac.jp

Accepted Oct 18, 2013; Epub ahead of print Jan 20, 2014

The human papillomavirus (HPV) can play an aetiological role in the development of anogenital epithelial lesions. The clinical spectrum includes condyloma acuminatum (CA), seborrhoeic keratosis (SK), bowenoid papulosis (BP), Bowen's disease (BD) and erythroplasia of Queyrat (EQ) (1). Of these, CA and SK are clinically and histopathologically benign lesions, whereas BP, BD and EQ are intraepithelial neoplasias. BP is frequently associated with HPV types with a high risk of malignancy, such as HPV types 16, 18, 31–35, 39, 42, 49 and 51–54 (2). BD also occurs in the anogenital area. The association of BD with HPV type 16 has been well established (3). BP and BD are histopathologically indistinguishable. CA is generally caused by HPV types 6 and 11, although many other genotypes have also been reported, including 2, 16, 18, 30–33, 35, 39, 41–45, 51–56 and 59, many of which are intermediate- or high-risk types (2). Genital SK is also regarded as a HPV-associated tumour (4). However, it has been indicated that SK that contain HPV are in fact CA (5). HPV type 6 was more frequently detected in these lesions (6).

The diagnosis of anogenital epithelial tumours is sometimes difficult. We studied HPV-positive anogenital epithelial tumours and herein discuss the usefulness of HPV typing.

MATERIALS AND METHODS

Biopsy specimens taken from 9 patients with anogenital epithelial lesions were used for this study. This project was approved by the Genome Ethics Committee of the Gunma University Graduate School of Medicine.

The biopsy specimens were fixed in 10% neutralised buffered formalin and embedded in paraffin wax. Formalin-fixed and

paraffin-embedded samples were then cut into 10 µm sections. The method used and PCR conditions have been described previously (7). DNA was extracted using Dexpat[®] (Takara, Kyoto, Japan). HPV PCR was performed with the L1C1/L1C2 consensus primers (8). The PCR products were subjected to direct sequencing after amplification. DNA extracted from a patient with BP, in which HPV type 56 was detected, was used as a control.

The catalysed signal amplification method (GenPoint System; Dako, Kyoto, Japan) (7) was used for the studies with formalin-fixed, paraffin-embedded specimens. The probe was a biotinylated high-risk HPV probe cocktail (GenPoint HPV; Dako) containing type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 HPV DNA. For the detection of HPV types 6 and 11, an HPV types 6/11 biotinylated DNA probe was used. The immunohistochemical analysis was performed using an anti-HPV antibody (K1H8; Dako) and the avidin-biotin complex method.

RESULTS

The clinical, histological and virological data of the 9 cases are summarised in Table I. Case 1 is representative, showing pigmented nodules in the labia majora, and the biopsy specimen revealed the presence of acanthosis, papillomatosis and full-thickness cytological atypia (Fig. 1a, b). Koilocytes were also seen in the upper epidermis (Fig. 1b). Cases 2, 3 and 8 presented with deeply pigmented multiple small nodules in the anogenital area (Fig. S1 Cases 2, 3 and 8 (a)¹).

Case 4a showed a dumbbell-shaped skin-coloured nodule on the dorsum of the penis (Fig. S1 Case 4 (a)¹). Case 5 showed a pigmented erythematous plaque on the labia majora (Fig. S1 Case 5 (a)¹). Case 6 presented with

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1784>

Table I. Clinical and virological features of human papillomavirus (HPV)-associated anogenital epithelial lesions

Case	Age, years/Sex	Diagnosis	Site	Clinical appearance	Histopathology				
					Atypia	Koilocyte	HPV type	ISH	IHC
1	54/F	Bowenoid papulosis	Labia majora	Multiple pigmented nodules	+	+	16	+	+
2	29/M	Bowenoid papulosis	Penis shaft	Multiple pigmented nodules	+	+	16	+	+
3	53/F	Bowenoid papulosis	Perianal area	Multiple pigmented nodules	+	+	67	+	+
4	40/M	Bowen's disease	Penis shaft	DSN	+	–	33	+	+
5	60/F	Bowen's disease	Labia majora	Pigmented plaque	+	–	16	+	–
6	69/F	Condyloma acuminata	Labia majora	Plaque	–	+	6	+	+
7	43/M	Condyloma acuminata/seborrhoeic keratosis	Penis shaft	Solitary pigmented nodule	–	+	43	ND	+
8	32/M	Condyloma acuminata/seborrhoeic keratosis	Penis shaft	Multiple pigmented nodules	–	–	6	–	–
9	35/M	Condyloma acuminata/seborrhoeic keratosis	Penis shaft	Pigmented plaque	–	–	6	–	–

ISH: *in situ* hybridisation; IHC: immunohistochemistry; DSN: dumbbell-shaped skin-coloured nodule; ND: not done.

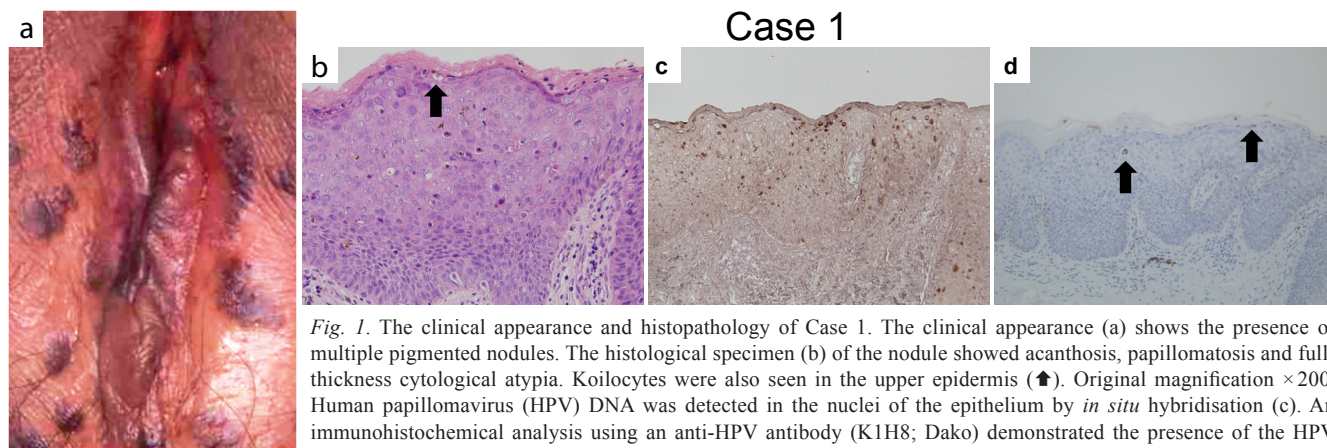


Fig. 1. The clinical appearance and histopathology of Case 1. The clinical appearance (a) shows the presence of multiple pigmented nodules. The histological specimen (b) of the nodule showed acanthosis, papillomatosis and full-thickness cytological atypia. Koilocytes were also seen in the upper epidermis (▲). Original magnification $\times 200$. Human papillomavirus (HPV) DNA was detected in the nuclei of the epithelium by *in situ* hybridisation (c). An immunohistochemical analysis using an anti-HPV antibody (K1H8; Dako) demonstrated the presence of the HPV capsid protein in the epithelium (▲) (d). Original magnification $\times 100$.

an erythematous plaque on the labia majora (Fig. S1 Case 6 (a)¹). Case 7 presented with a faintly pigmented small nodule on the ventral side of the penis (Fig. S1 Case 7 (a)¹), while Case 9 showed a pigmented plaque on the dorsum of the penile shaft (Fig. S1 Case 9 (a)¹). The biopsy specimens of Cases 1–3 showed similar histopathological findings. The epidermis showed acanthosis, papillomatosis and full-thickness cytological atypia (Fig. 1b and Fig. S1 Cases 2 and 3 (b)¹). Koilocytosis was seen in the epidermis of all 3 cases. Similarly, Cases 4 and 5 showed acanthosis, papillomatosis and full-thickness cytological atypia; however, no koilocytosis was seen (Fig. S1 Cases 4 and 5 (b)¹). The lesion in Case 6 revealed hyperkeratosis, papillomatosis and acanthosis without atypia. Koilocytes were seen in the upper epidermis in this case (Fig. S1 Case 6 (b)¹). The lesion of Case 7 showed mild hyperkeratosis and acanthosis without atypia. Koilocytes were also seen in the upper epidermis of this patient (Fig. S1 Case 7 (b)¹). The lesions of Cases 8 and 9 revealed acanthosis and basal pigmentation (Fig. S1 Cases 8 and 9 (b)¹). Pseudohorn cysts were also seen in Case 9.

The amplified PCR products showed a band at the expected position of 256 bp (data not shown). DNA sequencing revealed the L1 gene of HPV type 6, 16, 33, 67 and 43 (Table I). HPV type 16, 33 and 67 are considered to be in the high-risk group, while HPV types 6 and 43 are considered to be low-risk (9). HPV-positive cells were observed in the upper epidermis and stratum corneum of the lesion by *in situ* hybridisation (Table I, Fig. S1 Case 2 (a)¹) in 6 cases. The sample from Case 7 was unavailable for the analysis. HPV immunohistochemistry revealed that the viral protein was localised in the upper epidermis and stratum corneum (Table I, Fig. S1 Case 2 (b)¹).

DISCUSSION

The present study demonstrated the presence of HPV infection by 3 different methods, HPV typing with PCR,

in situ hybridisation and immunohistochemistry, in 9 anogenital epithelial lesions. Cases 1–3 were clinically and histopathologically diagnosed with BP, and virological studies demonstrated high-risk HPV types 16 and 67, both of which belong to the same species in the HPV classification. Cases 4 and 5 were diagnosed as BD both clinically and histologically. Case 4, in whom high-risk HPV type 33 was detected, histopathologically showed atypical cells. High-risk HPV type 16 was detected in Case 5. Case 6 presented with erythematous plaques, which were histologically and virologically compatible with CA. Cases 7 and 8 clinicohistopathologically showed pigmented small nodules without cellular atypia. Since some cases of CA are pigmented and cases on the penile shaft show less exophytic features (2, 10), differentiation between SK and CA is difficult. The lesions in the present patients were diagnosed as CA/SK caused by low-risk HPV types 6 and 43, respectively. In accordance with the previous reports, patient 9 was diagnosed to have genital CA/SK with HPV type 6 infection.

BP, BD and EQ are all forms of squamous intraepithelial neoplasia. However, the 3 types of lesions have different biological behaviours. In a previous study, Henquet (1) stated that the probability of malignant evolution is highest for EQ, intermediate for BD and low or non-existent for BP. This study clearly demonstrates that HPV infection occurs in various small epithelial lesions around the anogenital area. Since the clinical manifestations of these patients are diverse, it is often difficult to diagnose them based on their clinical features alone. Histological studies and HPV typing may be helpful for differentiating anogenital intraepithelial neoplasia from benign epithelial diseases.

REFERENCES

- Henquet CJ. Anogenital malignancies and pre-malignancies. *J Eur Acad Dermatol Venereol* 2011; 25: 885–895.
- Phillip H, McKee EC, Granter SR. *McKee's Pathology of the Skin* 3rd ed. Philadelphia: Mosby, 2005.
- Ikenberg H, Gissmann L, Gross G, Grussendorf-Conen EI,

- zur Hausen H. Human papillomavirus type-16-related DNA in genital Bowen's disease and in Bowenoid papulosis. *Int J Cancer* 1983; 32: 563–565.
4. Tardio JC, Bancalari E, Moreno A, Martin-Fragueiro LM. Genital seborrheic keratoses are human papillomavirus-related lesions. A linear array genotyping test study. *APMIS* 2012; 120: 477–483.
 5. Li J, Ackerman AB. "Seborrheic keratoses" that contain human papillomavirus are condylomata acuminata. *Am J Dermatopathol* 1994; 16: 398–405; discussion 6–8.
 6. Leonardi CL, Zhu WY, Kinsey WH, Penneys NS. Seborrheic keratoses from the genital region may contain human papillomavirus DNA. *Arch Dermatol* 1991; 127: 1203–1206.
 7. Shimizu A, Tamura A, Abe M, Motegi S, Nagai Y, Ishikawa O, et al. Detection of human papillomavirus type 56 in Bowen's disease involving the nail matrix. *Br J Dermatol* 2008; 158: 1273–1279.
 8. Yoshikawa H, Kawana T, Kitagawa K, Mizuno M, Yoshikura H, Iwamoto A. Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. *Jpn J Cancer Res* 1991; 82: 524–531.
 9. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004; 324: 17–27.
 10. Shimizu A, Tamura A, Nakatani Y, Shimizu N, Hoshino H, Ishikawa O. Pigmented plaque-type condyloma acuminatum associated with human papillomavirus type 6. *J Dermatol* 2012; 39: 860–861.