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Detection of Human Papilloma Virus Type 56 in Extragenital Bowen's Disease

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A case of Bowen's disease arising on the medial part of the first metatarsal bone of an 81-year-old Japanese woman is described. Histopathologically, proliferation of atypical cells was found throughout the epidermis. Electronmicroscopy revealed virus particles 40–50 nm in diameter in the nuclei of tumour cells at the granular cells just on or below the horny layer. Positive bands were obtained by polymerase chain reaction using a consensus primer of human papilloma virus L1 portion. Sequencing analysis of the amplified DNA revealed the same base sequences and homology as human papilloma virus 56. To the best of our knowledge, this case is the first report in which human papilloma virus 56 was found in a case of extragenital Bowen's disease. We consider it important to understand that human papilloma virus 56, often found in cervical lesions, can be detected in extragenital Bowen's diseases. ***Key words: extragenital Bowen's disease; human papilloma virus type 56; polymerase chain reaction; sequencing analysis.***

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Newly developed molecular biological techniques have enabled us to study viruses in the lesions of Bowen's disease (BD). We report here a case in which electronmicroscopy revealed virus particles in the nuclei of the tumour cells of Bowen's disease and polymerase chain reaction (PCR) revealed human papilloma virus (HPV), whose base sequence analysis identified it as HPV type 56.

CASE REPORT

An 81-year-old Japanese woman presented with keratotic lesion on the first left toe in April 1997, which she had first noticed about 8 years earlier. Though the lesion was persistent, she had ignored it as she thought it was a sore caused by her shoe. About 2 months before her first visit to our clinic, she noticed erythema and crust on the lesion with mild itching and tenderness. Meanwhile, she had another verrucous lesion on the left second finger about a year earlier, which was diagnosed as verruca vulgaris and was treated with cryosurgery.

Physical examination revealed a relatively well-demarcated erythema, 1.8×1.8 cm in size, at the plantar aspect of the medial part of the first metatarsal bone. There was a keratotic plaque in its centre with greyish scale and a pinkish crust (Fig. 1). She had a past history of asthma, hypertension, cataract and chronic inflammation of the bladder.

Laboratory findings, including complete blood count, biochemistry, and urinalysis, were within normal limits. However, anti-HTLV-1 antibody was positive. Haematoxylin-eosin (H & E) staining revealed irregularly arranged tumour cells with atypical nuclei throughout the epidermis, which disclosed hyperkeratosis, dyskeratotic cells and clumping cells, but immunohistological staining using polyclonal HPV (papilloma virus genus specific structural antigens, bovine (DAKO, Japan)) showed negative results. Electronmicroscopy revealed degenerated keratinocytes containing keratohyaline granules attached to tonofilaments in the horny layer. The keratinocytes were filled with electron-dense particles. Those particles were also seen outside the keratinocytes. They were closely aggregated, and partially arranged in linear and/or crystal patterns. The diameter of the particle was about 40–50 nm with both electron-dense and electron-low portions (Fig. 2). Those findings indicated that they were HPV particles. For causative HPV typing, we performed PCR and

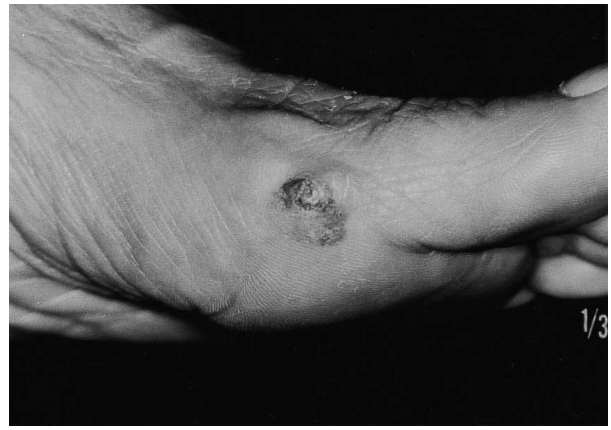

Fig. 1. A relatively well-demarcated erythema, 1.8×1.8 cm in size, at the first left toe. There was a keratotic plaque in its centre with greyish scale and pinkish crust.

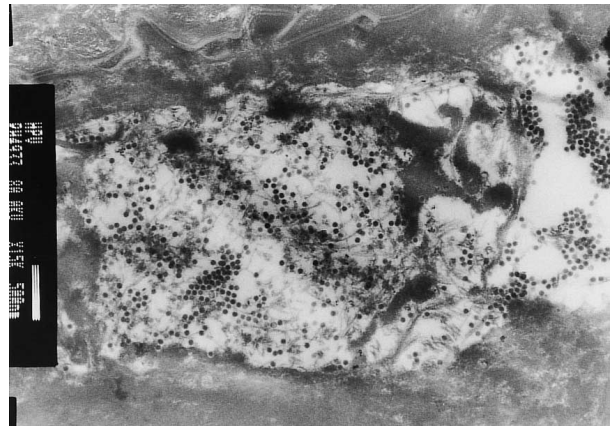

Fig. 2. Electronmicroscopy showed virus particles 40–50 nm in diameter in the keratinocytes just below the horny layer (15000×).

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sequencing analysis using the L1 consensus primer (L1C1; 5'-CGTAAACGTTTTCCCTATTTTTTT-3' and L1C2; 5'-CAATACA-GAGTATTTAGGGTA-3') as described previously (1). A positive band was obtained at 250 bp by PCR with L1 primer. In HPV 18 used as a positive control, positive band was seen at 250 bp. No bands of 250 bp were obtained in DNA from normal skin (Fig. 3). The amplified DNA by L1 primer was analysed for sequence using a HITACHI SQ5500 sequencer. The amplified DNA was substituted A with G at 5679 nt; the rest corresponded with the L1 portion of HPV 56 (5569–5818nt). The final diagnosis of BD was made on the basis of the clinical and histopathological findings. Removal of the lesion and skin grafting was made under local anaesthesia. No recurrence has been seen to date.

DISCUSSION

BD is a squamous cell carcinoma *in situ*, which has various initiators, such as ultraviolet ray exposure, intake of arsenium and HPV infestation.

Regarding HPV, as one of the initiators of BD, HPV 16 have been found with high incidence in the peri-anal and/or genital lesions (2). Stone et al. (3) and Kettler et al. (4) reported HPV 16 from foot, hand, palmar and arm lesions of extragenital BD. The other authors also reported HPV 16 in extragenital BD (5–9). Recently, several types of HPV (HPV-2, 3, 5, 18, 20, 33 and 34) have been reported in the lesions of BD (5, 6, 10–15) (Table I). Mitsuishi et al. (15) stated that HPV-31, 54, 58, 61, 62, and 73 have been identified in BD lesions. They concluded that HPVs related to mucosal lesions play an important role in development of BD of the hands. In the case described here, the result of detection of HPV antigens by immunohistological methods was negative, suggesting a possibility of "false negative". PCR is frequently used for this purpose by comparing the lengths of PCR products cut by restriction endonucleases. Besides these methods, there is another one to read the base sequences of DNA amplified by PCR. We used this method in this study. The DNA fragments obtained in our case were identified as HPV 56 with a point mutation at the L1 portion.

Table I. *Reports of HPV in extragenital Bowen's disease*

Reference	Site of the lesion	Type of HPV	Method
Lutzner M, et al. (1980) (11)	upper trunk and arms	5	immunofluorescence
	dorsal aspect of the second and third fingers of the right hand	5	
Pfister H & Haneke E (1984) (10)	the dorsum of the hand	2	DNA hybridization
Kawashima M, et al. (1986) (14)	periungual site of the left middle finger	34	blot hybridization and heteroduplex analysis
Stone MS, et al. (1987) (3)	second, third, and fourth toes of both feet	16	Southern blot hybridization
Ostrow RS, et al. (1987) (5)	arms	3	filter hybridization
	thumb	16	
Kettler AH, et al. (1990) (4)	medial soles	16	*in situ* DNA hybridization
	bilateral toes	16	
	volar upper arm	16	
	palm	16	
	palm	16	
	medial and volar finger	16	
Takahashi K & Kato T (1992) (6)	right sole	16	PCR and restriction fragment analysis
	left dorsum of the foot	18	
McGrae Jr JD, et al. (1993) (7)	second, third, fourth fingers	16	PCR
Shamanin V, et al. (1994) (12)	unknown	20	Southern blot hybridization
Sau P, et al. (1994) (8)	left middle finger	16	*in situ* hybridization
	left thumb	16	
	left middle finger	16	
	left ring finger	16	
Forslund O, et al. (1997) (9)	periungual area and proximal inter phalangeal joint of right middle finger, periungual area of the right thumb	16	PCR and DNA sequencing
	subungual area of left ring finger	16	
Mitsuishi T, et al. (1997) (15)	left palm	61	Southern blot hybridization, PCR, *in situ* hybridization, sequence analysis
	right first proximal digit	62	
	left third proximal digit	54	
	right second proximal digit	58	
	right third proximal digit	31	
	left second proximal digit	61	
	right third periungual	16	
	left third subungual	73	
Deguchi M et al. (1998) (13)	right temple	33	PCR, *in situ* hybridization
	right lower limb	33	

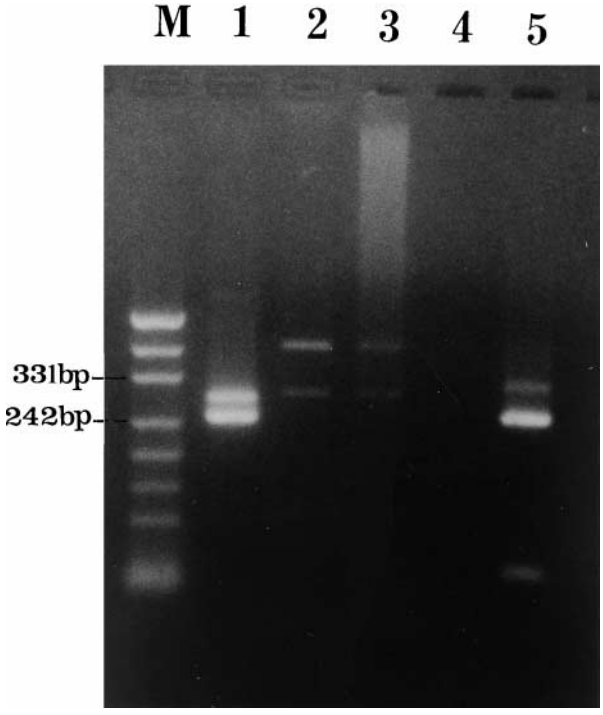

Fig. 3. Amplification of HPV using consensus L1 primer. Lane M: size marker, pUC19/HapII. Lane 1: total DNA obtained from the present case of Bowen's disease. Lane 2: total DNA obtained from a finger lesion of verrucous lesion. Lane 3: total DNA obtained from normal skin. Lane 4: dH_2O (no DNA). Lane 5: HPV type 18 (positive control).

It is well known that HPVs are closely involved with cervical cancer formation. Schiffman et al. (16) divided HPV into 2 major groups, high risk and low risk, according to histopathological features of PAP smears from cervical cancers. High risk group contained HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58, which are often detected from intraepithelial neoplasia and/or invasive cancers, while low risk group contained HPV types 6, 11, 42, 43 and 44, which are frequently found from benign specimens. According to this classification, HPV 56 found in our case belongs to the former.

HPV 56 was first detected in a cervical cancer by Lörincz et al. (17), and reported as a new type of HPV in 1989. They stated that HPV 56 was detected in invasive cancers of the cervix in and intraepithelial neoplasma, low incidence of normal cervical tissues and cervical condyloma. They added that HPV 56 was mostly related to HPV 30 and 45. The frequencies of HPV 56 detection have been reported as follows: 0.431% from normal cervical tissues, 2.20% from cervical condyloma and cervical intraneoplasia and 2.38% from invasive cancers of the cervix, which is 5.5 times as high as that from normal tissues (18). Our case is speculated that there may be some relationship between the subtypes of HPV and their degrees of carcinogenicity, metastasis and/or prognosis. Therefore, it is necessary to investigate the base sequences of HPVs whenever we encounter extragenital Bowen's diseases in order to clarify this problem.

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