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Human papillomavirus type 59 identified in a verrucous cyst of the flank

The verrucous cyst is a non-plantar epidermoid cyst with histopathological features of human papillomavirus (HPV) infection, including papillomatosis and hypergranulosis of the cyst lining. We report the first case of a verrucous cyst demonstrating not only the histopathological and immunohistochemical features of HPV infection, but also homology with HPV type 59 on HPV genotyping. A 28-year-old male developed a palpable mass in his right flank. Histological examination revealed an intradermal cyst lined by an acanthotic and papillomatous squamous epithelium with prominent keratohyaline granules and squamous eddies. The keratinocyte nuclei were positive for papillomavirus antigens on immunohistochemistry and HPV genotyping demonstrated a homology to HPV type 59, a high-risk genital type. Although we only experienced a single case with such a finding, we suggest that it may be necessary to subject patients with verrucous cysts to a closer follow up for better characterization of their clinical behavior.

Key words: epidermoid cyst, human papillomavirus 59, verrucous cyst

First described by Meyer *et al.* [1], the verrucous cyst is a non-plantar epidermoid cyst with histopathological features of human papillomavirus (HPV) infection, including papillomatosis and hypergranulosis of the cyst lining, and sometimes with the presence of koilocytes [1-6]. Immunohistochemical studies have revealed HPV antigens in a few cases [3, 6] and HPV genomes have been detected by polymerase chain reaction (PCR) [1, 4]; however, no specific HPV types have been detected so far. We report the first case of a verrucous cyst demonstrating not only the histopathological and immunohistochemical features of HPV infection, but also homology with HPV type 59 on HPV genotyping using a PCR-based DNA microarray system.

Case report

A 28-year-old Korean male visited the clinic complaining of a palpable mass in his right flank first discovered a month previously. On palpation, it was a pin-head sized, movable and non-tender papule and the overlying epidermis was grossly unremarkable without definite evidence of pore-like opening. The patient reported no prior history of viral warts or sexually transmitted diseases. Laboratory results, including a complete blood cell count and liver function test, were all within normal limits, and routine physical examination of the genital area revealed no gross abnormalities. A 3 mm-punch biopsy of the mass was performed under the clinical impression of epidermal cyst.

Histological examination with routine hematoxylin-eosin stain revealed a cyst located in the deep dermis, lined by an acanthotic squamous epithelium with areas of papillomatosis *(figure 1A)*. Hyperkeratosis was prominent especially at the tips of the papillomatous elevations, and large, prominent, irregular keratohyaline granules and pyknotic nuclei were noted in the granular layer. No definite koilocytotic features were seen *(figure 1B)*. The outer surface of the cyst was relatively smooth except for a focus where squamous eddies were present, creating a worrisome "infiltrative" appearance *(figure 1C)*. There was no pore-like opening into the epidermal surface even on serial sections. The overlying epidermis was histologically unremarkable. The surrounding stroma showed a mild chronic inflammation, and no adnexal structures were found near the cyst wall.

Immunohistochemistry using a polyclonal antibody against papillomavirus capsid antigen (DAKO, Carpinteria, CA, USA) was performed by the avidin-biotin procedure, and positive staining was seen in some of the pyknotic nuclei in the granular layer *(figure 1D)*.

HPV detection and genotyping was performed with extracted DNA using HPVDNAChip, a PCR-based DNA microarray system provided by Microarray Center, Biomedlab Co (Seoul, Korea). DNA was isolated from the paraffin-embedded tissue with a DNA isolation kit (Qiagen, Hilden, Germany), and target HPV DNA was amplified by a PCR with GP5d+/Gp6d+ primers (GP5d+, 5′-tttkttachgtkgtdgatacyac-3′; GP6d+, 5′-gaaahataaaytgyaadtcataytc-3′; k, g/ t; h, t/a/c; d, a/t/g; y, t/c). Beta-globin was amplified by PCR with PC03/PC04 primers (PC03, 5′-acacaactgtgttcactagc-3′; PC04, 5′-caacttcatccacgttcacc-3′) for internal control. The amplified DNA was labeled by Cy5-dUTP (NEN; Life Science Products Inc, Boston, MA, USA). The HPV-amplified product and beta-globin-amplified products were denatured by the addition of 3N sodium hydroxide solution, incubated for 5 minutes at room temperature, neutralized with 1 mol L^{-1} TRIS (tris-[hydroxymethyl]-aminoethane)–hydrochloric acid (pH 7.2) and 3N hydrochloric acid, and then finally cooled for 5

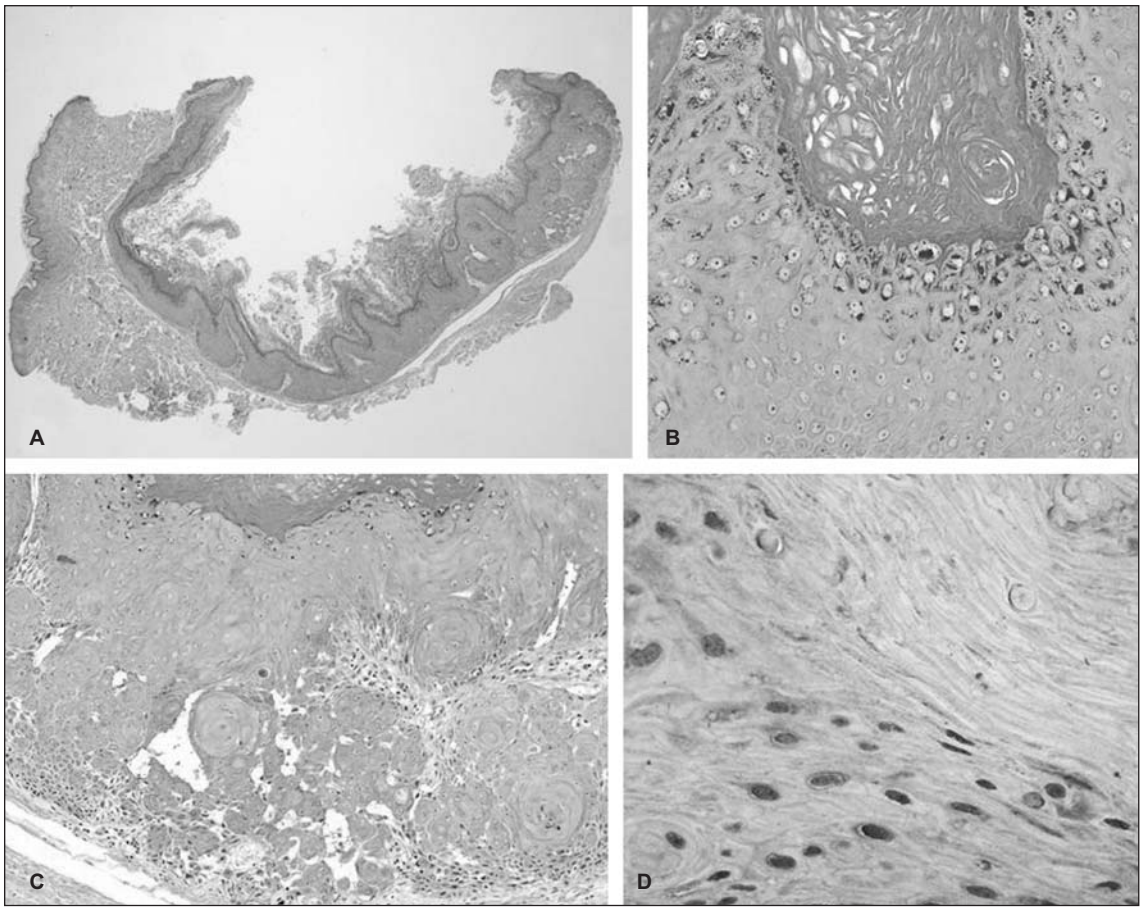

Figure 1. A) Scanning power view of the punch-biopsied specimen reveals a cyst located in the dermis, and lined by papillomatous and acanthotic squamous epithelium with overlying hyperkeratosis. **B)** A thickened granular layer with coarse keratohyaline granules are seen, with an overlying layer of hyperkeratosis. **C)** Squamous eddies are seen in the cyst wall [(A-C) Hematoxylin and eosin-stained sections, original magnification × 10 (A), × 200 (B) and × 100 (C)]. **D)** Immunohistochemistry for human papillomavirus capsid antigen shows positive staining in the keratinocyte nuclei (original magnification × 400).

minutes on ice. The samples were mixed with a hybridization solution made of 6X SSPE (saline-sodium phosphate-EDTA buffer; Sigma Chemical Co, St Louis, MO, USA) and 0.2% sodium dodecylsulfate, and applied to the DNA chip. Hybridization was performed at 40°C for 2 hours and then washed with 3X SSPE for 2 minutes, 1X SSPE for 2 minutes, and air-dried at room temperature. Hybridized HPV DNA was visualized using a DNA Chip Scanner (Scanarray lite; GSI Lumonics, Ottawa, Canada). Of the 22 types of mucocutaneous HPV tested, the type 59 probe hybridized with the fragment amplified from our specimen *(figure 2)*.

Discussion

HPV has been detected in a wide spectrum of mucocutaneous lesions, ranging from benign lesions such as verruca vulgaris to malignancies such as squamous cell carcinoma [7]. The number of diseases for which a role of HPV has been implicated is growing, and with the development of typing techniques such as *in situ* hybridization and polymerase chain reaction, specific HPV are being identified for

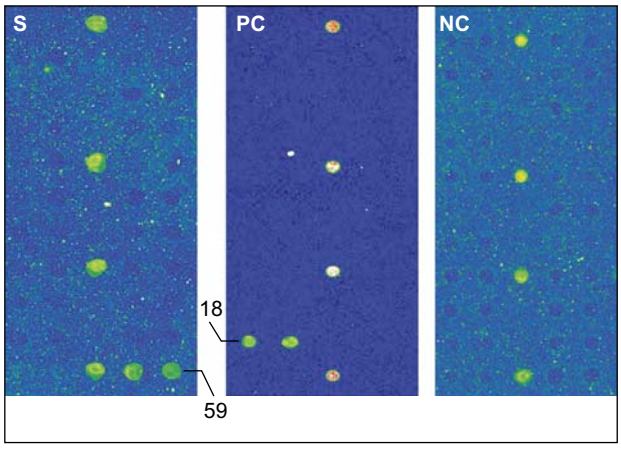

Figure 2. The HPV oligonucleotide microarray using probes against 22 types of mucocutaneous HPV. The four spots in the center of DNA chip are beta-globin. [S: patient sample; PC: positive control (HPV type 18-positive Hela cell line); NC: negative control (normal patient)].

each HPV-related disease entity. Epidermoid cysts arising in the palms and soles have also been shown to harbor HPV by immunohistochemistry and molecular studies, specifically types 57 and 60 [8-13], and HPV type 60 has been identified in an epidermoid cyst of non-palmoplantar location [14]. Furthermore, HPV has been demonstrated by PCR in verrucous cysts [1, 4], a rare entity first described by Meyer *et al*. in 1991; however, no specific types of HPV have been identified in such cysts.

Verrucous cysts differ from epidermoid cysts in the appearance of the cyst linings: acanthosis, papillomatosis, hypergranulosis with prominent keratohyaline granules, and hyperkeratosis, all being features reminiscent of HPV infection. Koilocytotic changes have also been sometimes reported, although they were not seen in our case. A striking feature in our case was the presence of squamous eddies in the cyst wall, resembling those seen in typical irritated seborrheic keratoses. Interestingly, five 'epidermoid cysts with seborrheic verruca-like cyst walls' have been previously reported [15] and described as showing acanthotic and papillomatous cyst linings, however, neither the presence of squamous eddies nor any relation to HPV were mentioned. Clinically, they are solitary lesions resembling epidermoid cysts and appearing most commonly on the face and back. They usually occur in adult patients, in contrast to common warts which are more prevalent in children or adolescents.

The verrucous cyst seen in our case showed histopathological features of HPV infection and also positivity for HPV capsid antigens by immunohistochemistry. However, this case is unique in that HPV genotyping demonstrated a homology to HPV type 59, a finding not previously mentioned. HPV type 59 is known to be a high-risk genital type, frequently detected in anogenital lesions such as cervical intraepithelial neoplasia and condylomata acuminate [16, 17]. HPV type 59 was initially cloned from a vulvar intraepithelial neoplasia and has been reported to show homology with HPV types 18, 45 and 39, which are also types associated with high-risk epithelial dysplasia. The detection of HPV type 59 in condylomata acuminata has been associated with immunosuppression [18]; however, our patient had no history or laboratory findings suggestive of immunosuppression.

Other than HPV-associated verrucous cysts and plantar epidermoid cysts, HPV has also been demonstrated in a verrucous trichilemmal cyst [6], and there are a few reports of molluscum contagiosum occurring in epidermoid cysts in the literature [19, 20]. The pathogenesis of cutaneous cysts associated with viral infection still remains obscure, however, it is postulated that the cysts may be induced by the virus *de novo*, or that the virus may infect pre-existing cysts [3-6]. Mechanical inclusion of HPV-containing epithelium into the dermis of weight-bearing areas has been suggested as a possible explanation for plantar epidermoid cysts [8, 9, 13], but this seems less likely to be the mechanism for cysts arising in non-plantar locations. Localization of the virus in the follicular ostia with subsequent obstruction and cyst formation, and epidermoid metaplasia of HPV-infected eccrine ducts have also been proposed as possible mechanisms for palmoplantar epidermoid cyst formation [21].

More than 10 years have elapsed since the verrucous cyst was first described in the literature [1], and although this may be an underreported entity, there have been no reports up to date implicating an aggressive behavior for these cysts, despite the proliferative features in the cyst walls which may appear alarming. However, as HPV type 59 is known to fall into the high-risk group in anogenital lesions, and as persistent infections of the skin with high-risk genital HPV types have been reported to represent a risk factor for non-melanoma skin cancer in non-immunosuppressed individuals [22], we suggest that it may be necessary to educate these patients about self-examination and to perform thorough in-office physical examinations on a regular basis for a better characterization of its clinical behavior. ■

References

1. Meyer LM, Tyring SK, Little WP. Verrucous cyst. *Arch Dermatol* 1991; 127: 1810-2.

2. Aloi F, Tomasini C, Pippione M. HPV-related follicular cysts. *Am J Dermatopathol* 1992; 14: 37-41.

3. Elston DM, Parker LU, Tuthill RJ. Epidermoid cyst of the scalp containing human papillomavirus. *J Cutan Pathol* 1993; 20: 184-6.

4. Soyer HP, Schadendorf D, Cerroni L, *et al*. Verrucous cysts: histopathologic characterization and molecular detection of human papillomavirus-specific DNA. *J Cutan Pathol* 1993; 20: 411-7.

5. Reis MD, Tellechea O, Baptista AP. Verrucous cyst. *Eur J Dermatol* 1998; 8: 186-8.

6. Misago N, Narisawa Y. Verrucous trichilemmal cyst containing human papillomavirus. *Clin Exp Dermatol* 2005; 30: 38-9.

7. Lutzner MA. The human papillomaviruses. A review. *Arch Dermatol* 1983; 119: 631-5.

8. Egawa K, Inaba Y, Ono T, *et al*. "Cystic papilloma" in humans? Demonstration of human papillomavirus in plantar epidermoid cysts. *Arch Dermatol* 1990; 126: 1599-603.

9. Kitasato H, Egawa K, Honda Y, *et al*. A putative human papillomavirus type 57 new subtype isolated from plantar epidermoid cysts without intracytoplasmic inclusion bodies. *J Gen Virol* 1998; 79: 1977-81.

10. Egawa K, Kitasato H, Honda Y, *et al*. Human papillomavirus 57 identified in a plantar epidermoid cyst. *Br J Dermatol* 1998; 138: 510-4.

11. Matsukura T, Iwasaki T, Kawashima M. Molecular cloning of a novel human papillomavirus (type 60) from a plantar cyst with characteristic pathological changes. *Virology* 1992; 190: 561-4.

12. Ashida M, Ueda M, Kunisada M, *et al*. Protean manifestations of human papillomavirus type 60 infection on the extremities. *Br J Dermatol* 2002; 146: 885-90.

13. Kashima M, Takahama H, Baba T, *et al*. Detection of human papillomavirus type 57 in the tissue of a plantar epidermoid cyst. *Dermatology* 2003; 207: 185-7.

14. Lee S, Lee W, Chung S, *et al*. Detection of human papillomavirus 60 in epidermal cysts of nonpalmoplantar location. *Am J Dermatopathol* 2003; 25: 243-7.

15. Rahbari H. Epidermoid cysts with seborrheic verruca-like cyst walls. *Arch Dermatol* 1982; 118: 326-8.

16. Rho J, Roy-Burman A, Kim H, *et al*. Nucleotide sequences and phylogenetic classification of human papillomavirus type 59. *Virology* 1994; 203: 158-61.

17. Bryan JT, Tekchandani J, Schroeder JM, *et al*. Propagation of human papillomavirus type 59 in the athymic mouse xenograft system. *Intervirology* 2000; 43: 112-8.

18. Brown DR, Schroeder JM, Bryan JT, *et al*. Detection of multiple human papillomavirus types in condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J Clin Microbiol* 1999; 37: 3316-22.

19. Aloi F, Pippione M. Molluscum contagiosum occurring in an epidermoid cyst. *J Cutan Pathol* 1989; 12: 163-5.

20. Fellner MJ, Osowsky MJ. Molluscum contagiosum occurring in an epidermoid inclusion cyst. *Int J Dermatol* 1979; 18: 160-1.

21. Egawa K, Egawa N, Honda Y. Human papillomavirus-associated plantar epidermoid cyst related to epidermoid metaplasia of the eccrine duct epithelium: a combined histological, immunohistochemical, DNA-DNA *in situ* hybridization and three-dimensional reconstruction analysis. *Br J Dermatol* 2005; 152: 961-7.

22. Iftner A, Klug SJ, Garbe C, *et al*. The prevalence of human papillomavirus genotypes in nonmelanoma skin cancers of nonimmunosuppressed individuals identifies high-risk genital types as possible risk factors. *Cancer Res* 2003; 63: 7515-9.

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