Vulvar squamous papillomatosis and human papillomavirus infection. A polymerase chain reaction study

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Abstract. Squamous papillae of the vulvar vestibule and introitus are quite a common clinical finding, however their origin is uncertain. They were formerly described as a normal variant of the mucosal epithelium, but recently they have been attributed to human papillomavirus (HPV) infection. Eight women with clinical findings compatible with a diagnosis of vulvar squamous papillomatosis were studied. All were free of other clinically evident HPV-related diseases. Vulvar scrapes and biopsy specimens were collected and used for DNA extraction and microscopic examination. DNA extracted from vulvar scrapings and from paraffin-embedded tissue was subjected to polymerase chain reaction (PCR). The reactions were performed with two sets of primers designed for the amplification of numerous HPV genotypes including those most commonly encountered in the genital area. Histological examination failed to reveal clear-cut signs of HPV infection in any subject. The PCR on the DNA extracted from vulvar scrapings revealed HPV infection in two cases. PCR performed on the DNA extracted from the paraffin-embedded tissue failed to detect HPV-DNA in any case. A 6-month follow-up showed no changes in the lesions. These results along with literature data, which is clearly inconsistent, indicated that the presence of HPV is coincident to, rather than causal of, vulvar squamous papillomatosis lesions. Patients with symmetrically distributed, long-standing vulvar papillae should, therefore, be carefully evaluated before starting therapy.

Key words: Vulvar squamous papillomatosis — Vulvar diseases — Human papillomaviruses — Polymerase chain reaction

Examination of the female external genitalia sometimes reveals many papular or frond-like lesions symmetrically distributed on the inner face of the labia minora, vulva vestibule and/or introitus. This condition, sometimes associated with pruritus and/or burning, is known in the literature as hirsuties papillaris vulvae [1, 19], hirsutoid papillomas of the vulva [11, 9], benign squamous papillomatosis [14], vulval squamous papillomatosis [23, 10], micropapillomatosis labialis [2] and squamous vestibular micropapilloma [16]. The abundance of names reflects the uncertain origin of this condition, which will be referred to as vulvar squamous papillomatosis (VSP) in this paper. Some authors regard VSP as a physiological variant of mucosal epithelium [1, 7, 9, 14, 16] sometimes enhanced by chronic inflammation [6]. Others, on the basis of microscopic examination and immunocytochemical and DNA hybridization studies, consider that VSP could be one of several clinical expressions of genital human papillomavirus (HPV) infection [3, 10, 22, 23]. However, it has not been convincingly demonstrated that HPV plays a clear role in the pathogenesis of VSP.

In this study we used a method based on polymerase chain reaction (PCR) to investigate the presence of HPV-DNA in VSP lesions of eight patients clinically free of other HPV-related diseases.

Materials and methods

Patients

From all the female patients referred to our clinic for suspected vulvar condylomata acuminata, we selected eight (age range 22–27 years) with vulvar lesions compatible with a diagnosis of VSP (Table 1). At the first examination, the external genitalia and the perianal area were observed before and after the application of 5% acetic acid. All patients were referred to the gynecology clinic of Siena University for a complete gynecological examination, including colposcopy and PAP-smear. A detailed medical history of each patient, mainly regarding the onset and evolution of the lesions and earlier sexually transmitted diseases, was obtained. The sex partners of all patients were examined to exclude clinical and subclinical manifestations of genital HPV infection. All patients were invited to attend monthly follow-up for a period of at least 6 months.

Clinical samples

After informed consent had been given by the patients, epithelial and tissue biopsy specimens of pathological genital mucosa were...
Table 1. HPV-DNA detection by PCR in cell scrapes (Scr.) and paraffin-embedded tissue (Bio.)

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)</th>
<th>Previous STD</th>
<th>PCR Scr.</th>
<th>PCR Bio.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>No</td>
<td>Neg</td>
<td>Neg</td>
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</tr>
<tr>
<td>8</td>
<td>26</td>
<td>No</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

STD, Sexually transmitted diseases; PCR, polymerase chain reaction; CA, condyloma acuminatum

Obtained. Epithelial samples were taken by gently scraping the mucosa with the edge of a scalpel. The scalpel was immediately rinsed in a lysis solution containing 0.5% SDS, 10 mM Tris-HCl, 10 mM EDTA and 100 μg/ml proteinase K, and the solution was stored at -20 °C until further processing.

Biopsies were performed under local anaesthesia, the material was fixed in absolute ethanol and processed as for routine histological examination. Serial sections were used for standard haematoxylin and eosin staining and DNA extraction.

DNA purification and PCR

DNA extraction from epithelial scrapings was performed by the standard phenol-chloroform method [13]. Briefly, the samples were incubated at 37 °C for 1 h, and deproteinized by extraction with phenol, phenol-chloroform and chloroform. The nucleic acids were then precipitated with absolute ethanol. The DNA was then resuspended in 200 μl sterile water. We used 50 μl aliquots for PCR amplification without nucleic acid quantification. DNA extraction from paraffin sections was performed as described by Greer [8]. The presence of sufficient DNA for amplification and of DNA polymerase inhibitors was checked by amplifying 50 μl of the extracted DNA with two primers (AR1-AR2) spanning the DRB1 region of the HLA genes, as described by Ratti [17].

Amplification of HPV DNA was performed using two different sets of primers. By computer analysis [5] we selected two near-consensus primers, namely 2DX (5'-GGTGGAACCGA-G-3') and 2SX (5'-CCTGAATACTTTCGTTTTA-3'), framing about 1000 base pairs between the URR region and the E1 gene of HPV types 6, 11, 16, 18, 31 and 33. Amplifications were carried out as described by Saiki et al. [20] with minor modifications. Briefly, they were performed in 0.5 ml tubes in 100 μl of reaction mixture containing sample DNA, 50 mM KCl, 10 mM Tris-HCl, pH 8.8, 1.5 mM MgCl2, 0.1% Triton X-100, 0.2 mM dNTP, 50 pmol of each primer and 2 units of thermostable Taq-DNA polymerase (Promega). The solution was overlaid with several drops of mineral oil and amplification performed in a DNA thermal cycler (Perkin-Elmer, Cetus) as follows: 7 cycles comprising incubations at 95 °C for 40 s, 37 °C for 40 s and 72 °C for 2 min, followed by 30 cycles comprising incubations at 95 °C for 40 s, 50 °C for 40 s and 72 °C for 2 min. After amplification, 10 μl of each reaction mix was analyzed by electrophoresis in 1% agarose gel. DNA bands were stained with ethidium bromide and photographed under UV light. The PCR was developed and optimized using a set of HPV genomes cloned in bacterial plasmid vectors. Using the above conditions it was possible to amplify a large number of HPV types (HPV 1, 2, 3, 4, 6, 11, 16, 18, 31 and 33) including those most commonly encountered in the genital tract [15]. The PCR on DNA extracted from sections was checked on several paraffin-embedded condylomata acuminata. Under the above conditions we detected HPV-DNA in more than 90% (19/20) of cases. Six type-specific internal probes were selected for HPV 6, 11, 16, 18, 31 and 33, and used in a dot-blot assay to identify the amplified products. Briefly, 5 μl of each amplification mix was spotted onto nylon membranes and hybridized in 6 x SSC (1 x SSC is 0.15 M NaCl 0.015 M Na citrate) at 54 °C with 32P terminal labeled type-specific probes as described elsewhere [15]. Amplifications were also performed with two different primers (GP5–GP6), capable of amplifying many HPV types, using the method described by Snijders [21]. Oligonucleotides were synthesized with an applied Biosystem DNA synthesizer mod. 380 B, using reagents and methods recommended by the manufacturers.

Results

Clinical examination

Clinical examination revealed eight patients with small papular or frond-like soft pink papillae on the inner surface of the labia minora, vulva introitus or vulva vestibule. The papillae were symmetrically distributed and clearly visible to the naked eye (Fig. 1). In two cases the acetic acid test induced diffuse mucosal whitening. These subjects complained of nonspecific inflammation, with pruritus and burning. All patients claimed that the papillary lesions had persisted unmodified, to the best of their knowledge. Patient 3 (Table 1) had been treated for condylomata acuminata several months earlier in the gynaecology clinic of Siena University while the others had a negative history for sexually transmitted diseases.

![Fig. 1. Typical clinical appearance of vulvar squamous papillomatosis (case no. 6). Note the papillae all over the inner surface of the labia minora](image-url)