Detection of human papillomavirus types in balanitis xerotica obliterans and other penile conditions

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Abstract
Objectives—To determine the prevalence of human papillomavirus (HPV) types 6, 11, 16 and 18 in foreskin biopsies from patients with balanitis xerotica obliterans (BXO) and other penile conditions.

Materials and methods—Foreskin biopsy specimens from 24 patients with penile lesions and 5 control patients were analysed by type-specific polymerase chain reaction (PCR).

Results—HPV6 or HPV16 were not detected in patients with BXO. HPV6 was detected in 2 controls.

Conclusions—Genital papillomaviruses do not have a strong association with BXO.

Materials and methods
Materials Foreskin biopsy specimens had been collected between September 1991 and March 1992 from 10 patients with a clinical and/or histological diagnosis of BXO presenting to a genitourinary unit in Birmingham. Referral was from the entire unit and had been for circumcision. The specimens had been stored in formalin-saline at −20°C. Biopsy specimens from patients with other penile skin disorders were collected over the same period. Five “normal” foreskins from adult patients undergoing routine circumcision for religious reasons were obtained from Leicester as control tissue.

DNA extraction Approximately 2 mm³ of tissue was cut from each biopsy using a sterile scalpel. This was suspended in 1 ml phosphate-buffered saline and freeze-thawed three times. After centrifugation at low speed (7000 g) the supernatant was discarded. The pellet was then homogenised in 1 ml of tissue digestion buffer (100 mM NaCl, 25 mM EDTA, 0.5% sodium dodecyl sulphate, 10 mM Tris-HCl pH 8) using a sterile mortar and pestle. The homogenate was then transferred to a 1.5 ml Eppendorf tube and digested with 10 µl of proteinase K (20 mg/ml) at 37°C. Digestion was allowed to proceed until particulate material was not macroscopically visible. This was usually overnight but could take up to 5 days; which would require the addition of fresh proteinase K at 24 hour intervals. The digested material was then extracted once with chloroform:isoamylalcohol (ratio 24:1) and nucleic acid precipitated in 100% ethanol at 4°C. After centrifugation the nucleic acid pellet was resuspended in 100 µl sterile water for amplification by polymerase chain reaction (PCR).

Polymerase chain reaction
Human papillomavirus (HPV)-specific PCR Oligonucleotide primers were synthesised from published sequences for HPV types 6, 11, 16 and 18.3 These sequences were derived from the E6 region and are shown in table 1.

Table 1 Oligonucleotide primer sequences used in the PCR amplification (from reference 5). All primers are annotated with reference to Genbank sequences and are written in a 5' to 3' orientation

<table>
<thead>
<tr>
<th>HPV type</th>
<th>5' Primer</th>
<th>3' Primer</th>
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<tbody>
<tr>
<td>HPV 6</td>
<td>TCT ATC TAT GCA TAC GTT GC</td>
<td>CCA TTT TAT ATA TGA TTG GC</td>
</tr>
<tr>
<td>HPV11</td>
<td>TCT TTG TTT GTA CAC GTT GC</td>
<td>GCA GTA TTT GGA TTG GC</td>
</tr>
<tr>
<td>HPV16</td>
<td>CAG GAC CCA CAG GAC GGA CC</td>
<td>CCC AGC CAC CTG GCC AGC TA</td>
</tr>
<tr>
<td>HPV18</td>
<td>GCT TTG AGG ATC CAA CAC GGC</td>
<td>GGT CAC GGT AGG CAC GAC GT</td>
</tr>
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Extracted DNA (0.5 μl) was amplified using the appropriate primers in an Amplitaq assay (Perkin-Elmer, Norwalk, USA) using the recommended buffer and reagent concentrations, and 5 units of Amplitaq polymerase. Appropriate positive controls, consisting of HPV insertion sequences derived from plasmids (kindly supplied by Professor E.M. de Villiers of the Deutsches Krebsforschung-Zentrum, Heidelberg) were used in all amplification runs. A negative control, sterile water, was also included and precautions were taken to minimise the risks of DNA carryover. After an initial "hot-start" of 94°C for 10 minutes, different cycling parameters were used for specific virus types. For amplification of HPV6 and HPV11 sequences, thirty cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for one minute were followed by a final extension at 72°C for 10 minutes. For amplification of HPV16 and HPV18 sequences the thermal cycling protocol was thirty cycles of 95°C for 30 seconds, 68°C for 30 seconds and 72°C for one minute, with a final extension at 72°C for 10 minutes. All amplifications were undertaken in a Geneamp 9600 cycler (Perkin-Elmer, Norwalk, USA). Amplification products were visualised by electrophoresis in 2% agarose gels and ethidium bromide staining with ultraviolet illumination.

All specimens were subjected to amplification of β-globin genes using standard primer sequences.

Results
Figures 1 and 2 show the results of positive (typeable) specimens. All the specimens were β-globin PCR positive. Table 2 summarises the results and histological diagnoses. It can be seen that none of the BXO specimens had detectable HPV sequences. The genital warts had detectable HPV as might be expected but interestingly only one was a common genital type. One of the cases of non-specific inflammation had detectable HPV but without following up clinical information we cannot assess any pathological significance. Two of the five control specimens were positive for HPV6 only (data not shown).

Discussion
The evidence for an infectious aetiology for BXO is predominantly anecdotal. There is some support for the hypothesis, however, from studies that suggest there is chronic antigen stimulation in the disease. The association with squamous carcinoma might suggest an agent that had oncogenic potential. Much evidence has accumulated that specific HPV
types have this potential. One of these is HPV16 but this was not found in our patients using a very sensitive technique. The numbers are small but statistically the prevalence of HPV types in the BXO and non-BXO groups is different (p < 0.05).

The prevalence of genital human papillomavirus infections in the adult male population is not known with any great precision but studies in a subset, those that are sexually active have suggested that between 20 and 45% of men would be expected to carry genital papillomaviruses.9,10 This figure is higher in contacts of women with known cervical HPV infection.11 We do not know the sexual histories of the patients enrolled in this study but it is clear that the prevalence in our control patients is consistent with other studies but the prevalence in patients with BXO is significantly less. This would negate a significant or consistent role for human genital papillomaviruses in balanitis xerotica obliterans. The search for an infectious agent should continue.

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