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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.**

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Keywords: Balanitis xerotica obliterans; Papillomavirus

Introduction

Balanitis xerotica obliterans (BXO), also known as lichen sclerosis of the penis is an unusual condition that predominantly affects young adults and children. It is first manifest as white atrophic patches which may be mildly itchy. The plaques form bands which will eventually start to constrict the penis. The patient may suffer recurrent episodes of balanitis and eventually circumcision is often necessary.[1] Squamous cell carcinoma has also been reported as a rare complication of the condition.[2 3] The aetiology of this condition is unknown although genetic, autoimmune, hormonal and infectious mechanisms have been proposed.[4] The search for a possible infectious agent has led us to examine the presence of known genital viruses in this condition. We present in this paper the results of screening for specific genital papillomaviruses, a possibility raised by the association of these viruses with squamous carcinoma.

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 formol-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 mm^3 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)-specifc PCR Oligonucleotide primers were synthesised from published sequences for HPV types 6, 11, 16 and 18.[5] These sequences were derived from the E6 region and are shown in table 1.

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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

HPV type	*5′ Primer*	*3′ Primer*
HPV 6	TCT ATC TAT GCA TAC GTT GC	CCA TTT TGT ATA TGA TTG GT
HPV11	TCT TTC TTT GCA CAC TCT GC	GAA GTA TTT TGA TTT ATT GG
HPV16	CAG GAC CCA CAG GAC CGA CC	CCC AGC CAC CTG GCC AGC TA
HPV18	GCT TTG AGG ATC CAA CAC GG	GGT CAC GGT AAG CAC GAC GT

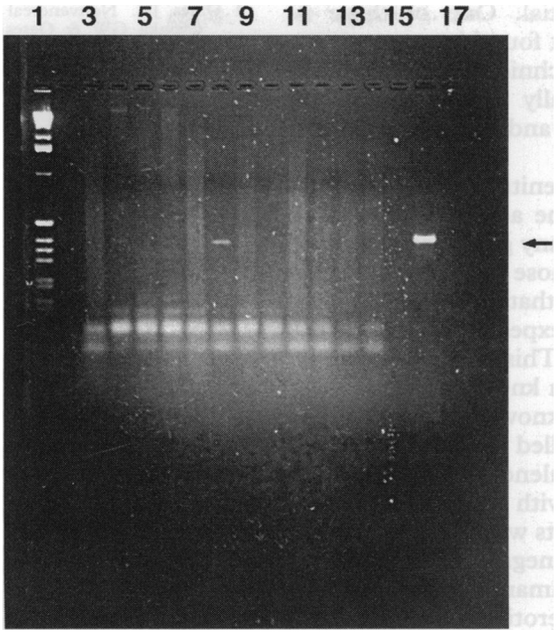

Figure 1 PCR amplification of HPV6 sequences from patients with penile lesions. Molecular weight markers (1kb ladder) is shown in lane 1, lane 8 is a positive specimen (specimen 10) and lane 16 a positive control (HPV6 plasmid). Lanes 3–7 and 9–14 are negative specimens. A negative control is present in lane 17.

Table 2 Histological diagnoses and HPV types in 24 patients with penile conditions. Specimens that were not typed were positive by the HPV-general PCR but not by type-specific PCR

Patient number	*Histological diagnosis*	*HPV type*
1	No diagnosis available	No HPV
2	Balanitis xerosis obliterans	No HPV
3	Balanitis xerosis obliterans	No HPV
4	Non-specific balanitis	No HPV
5	Balanitis xerosis obliterans	No HPV
6	Genital warts	HPV not typed
7	Balanitis xerosis obliterans	No HPV
8	No diagnosis available	No HPV
9	Non-specific inflammation	No HPV
10	Genital warts	HPV6
11	Balanitis xerosis obliterans	No HPV
12	Balanitis xerosis obliterans	No HPV
13	Herpesvirus infection	No HPV
14	Non-specific infection	No HPV
15	Genital warts	HPV not-typed
16	Balanitis xerosis obliterans	No HPV
17	Bowenoid papulosis	No HPV
18	Non-specific inflammation	HPV16
19	Balanitis xerosis obliterans	No HPV
20	Non-specific inflammation	No HPV
21	Balanitis xerosis obliterans	No HPV
22	Non-specific inflammation	No HPV
23	Balanitis xerosis obliterans	No HPV

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 DeutschesKrebsForschung-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.

HPV general PCR Biopsy specimens that were negative by HPV-specific PCR were subjected to a PCR amplification with primers that would hybridise to sequences in most HPV types (though not HPV16).[6]

All specimens were subjected to amplification of β-globin genes using standard primer sequences.[7]

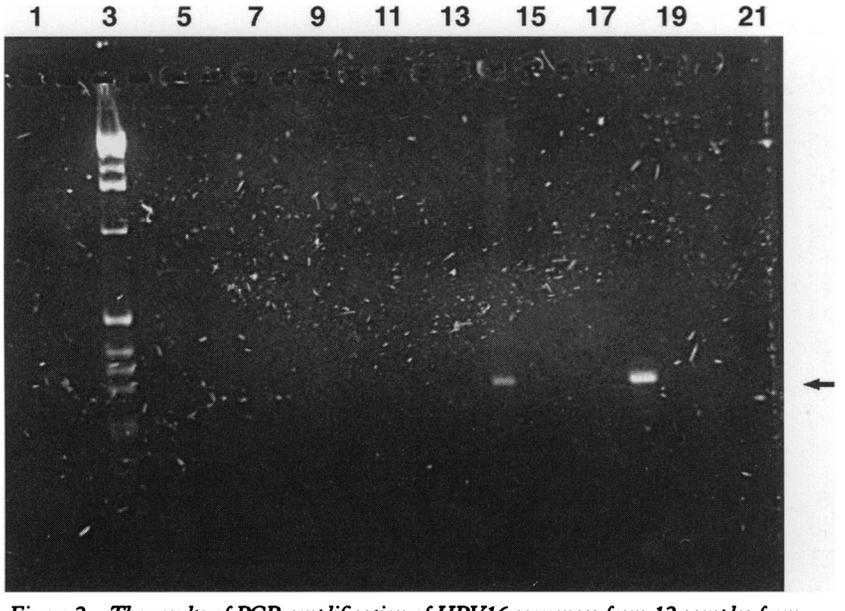

Figure 2 The results of PCR amplification of HPV16 sequences from 12 samples from patients with penile lesions. Molecular weight markers (1kb ladder), are shown in lane 3, lanes 4–13 and 15 are negative specimens, lane 14 (specimen 18) a positive specimen. A positive control is shown in lane 18, a negative in lane 1.

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 follow 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.[8] 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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