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article

Detection of human papillomavirus DNA on the fingers of patients with genital warts

C Sonnex, S Strauss, J J Gray

Objective: To determine whether patients with genital warts carry human papillomavirus (HPV) DNA on their fingers.

Methods: 14 men and eight women with genital warts had cytobrush samples taken from genital lesions, finger tips, and tips of finger nails. Samples were examined for the presence of HPV DNA by the polymerase chain reaction.

Results: HPV DNA was detected in all female genital samples and in 13/14 male genital samples. HPV DNA was detected in the finger brush samples of three women and nine men. The same HPV type was identified in genital and hand samples in one woman and five men.

Conclusion: This study has identified hand carriage of genital HPV types in patients with genital warts. Although sexual intercourse is considered the usual mode of transmitting genital HPV infection, our findings raise the possibility of transmission by finger-genital contact.

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Keywords: human papillomavirus; polymerase chain reaction; fingers; genital warts

Introduction

Genital human papillomavirus (HPV) infection is considered to be acquired predominantly through sexual intercourse.¹ A number of studies, however, have suggested that non-sexual transmission may occasionally occur among adults²⁻⁴ and, in addition, there are case reports of children with ano-genital warts who were thought to have acquired their infection from relatives with hand warts.^{5,6} The purpose of this study was to determine whether patients with genital warts carry HPV on their fingers and if so, whether this is of the same type as found in the genital lesions.

Methods

Fourteen men and eight women with genital warts attending a department of genitourinary medicine were recruited into the study. Genital lesions were gently brushed with a cytobrush moistened with phosphate buffered saline (PBS, pH 7.3) and the brush then placed in PBS before transport to the laboratory. Separate brush samples were also obtained from patients' finger tips and tips of finger nails. In an attempt to reduce the risk of inadvertent sample contamination, gloves were worn when obtaining genital brushings and then discarded before finger brushing. In addition, cytobrushes were stored in a sealed bag and only came into contact with the site being sampled.

In the laboratory, all samples were then vortexed and centrifuged at 11 000 *g* for 3 minutes. The supernatant was discarded and the cell pellet was resuspended in Cetus buffer (TRIS-HCl 10 mM, pH 8.3; KCl 50 mM; MgCl₂ 2.5 mM; gelatin 0.1 mg/ml; P40 0.45%; Tween 20 0.45%). Proteinase K (60 µg/ml) was added followed by heating of the sample to 55°C for 1 hour and boiling at 95°C for 10 minutes. The samples were then stored on ice for 10 minutes and used for analysis or stored at -20°C.

The polymerase chain reaction (PCR) was performed to detect HPV DNA. Concentrations of MgCl₂, dNTPs, and primers as well as annealing temperatures and elongation times were optimised for all PCRs.

The initial amplification was performed using the L1 consensus degenerate primers MY09/MY11, initially described for the detection of genital HPVs⁷ but also widely used to analyse skin lesions. The reaction was performed in a 50 µl reaction mix and produced an amplicon of approximately 450 bp.

The second round of amplification was performed on an aliquot of the first round product with the general primers GP5+/GP6+,⁸ located within the MY09/MY11 primers, for the detection of a broad spectrum of HPV genotypes in genital samples with a increased sensitivity, yielding an amplicon of 150 bp.

A seminested PCR was used to detect a broad spectrum of cutaneous HPV genotypes. The first round of PCR was performed using the HPV2/B5 primers⁹ in a 50 µl reaction mix, expecting an amplicon of 650 bp size. The second round of amplification was performed using the NPR2/B5 primers,¹⁰ in a 50 µl reaction mix yielding an amplicon of 400 bp.

Finally, 10 µl of PCR product were electrophoresed through a 3% agarose gel in TAE containing ethidium bromide (0.4 µl of 10 µg/ml).

Genital samples which were negative for HPV DNA and all hand samples were tested for the presence of β globin DNA using the GH20 and PC04 primers to determine the presence of cellular DNA.¹¹

The HPV type of the MY09/MY11 PCR products was determined by restriction fragment length polymorphism (RFLP) with the restriction enzymes Rsa I, Pst I, and Hae III.¹² DNA sequencing of the GP5+/GP6+ PCR product¹³ was performed on samples which

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Table 1 Human papillomavirus (HPV) detection according to site and HPV type

Penile HPV type	β globin	Hand HPV type	β globin
<i>Men:</i>			
31	NT	NS	+
6	NT	6	+
NS	NT	ND	-
NS	NT	ND	+
6	NT	ND	+
16+6	NT	66	+
6	NT	6	+
6	NT	6	+
16	NT	N.S.	+
11	NT	11	+
6	NT	6	+
ND	-	ND	-
6	NT	ND	+
11	NT	NS	NT
Vulval HPV type	β globin	Hand HPV type	β globin
<i>Women:</i>			
6	NT	ND	-
NS	NT	ND	-
NS	NT	ND	+
NS	NT	ND	-
6	NT	31	+
6	NT	ND	-
6	NT	6	+
6	NT	66	+
(cervix, type II)			

NT = not tested; ND = not detected; NS = non-specified.

could not be typed by RFLP or only gave a product after the second round of amplification.

Results

HPV DNA was detected in all female genital brush samples and in 13/14 male genital samples. HPV DNA was detected in the finger brush samples of three women (identical to the genital HPV type in one woman) and nine men (identical to the genital HPV type in five men). β Globin DNA was absent from the one HPV DNA negative penile sample and from six of the 10 HPV DNA negative hand samples, suggesting inadequate sampling. The results are summarised in table 1.

Discussion

This study has documented hand carriage of HPV DNA in 38% of women and 64% of men with genital warts. In total, 27% of patients had identical HPV types detected in both genital and hand samples. These findings obviously raise the possibility that patients with genital warts may transfer genital HPV types to their sexual partners by finger-genital contact. Previous clinical studies have also suggested possible hand transmission of genital HPV infection. Fairley *et al* failed to identify genital HPV infection in a group of young virginal women using tampons for specimen collection¹⁴ whereas studies using vulval swabs have documented genital HPV carriage.^{3 4 15} Similarly, Bauer *et al* detected HPV DNA in three vulval swabs but not cervical scrapes from 15 virginal women.¹⁶

The possibility of hand transmission is also suggested by the reports of children with anogenital warts who were considered to have

acquired their infection from close family members with hand warts or by autoinoculation from their own hand warts.^{5 6 17-19}

Interestingly, HPV type 66 was identified from hand samples of two patients but from no genital samples. This is a rare HPV type previously detected in cervical carcinoma, high grade cervical dysplasia, and from a cervical specimen of a woman with normal cervical cytology.^{20 21} A number of specimens contained HPV DNA of unspecified (that is, currently unrecognised) type and are undergoing further analysis.

This was a small, preliminary study and further larger studies are required, ideally including a control group of patients with no history of genital HPV infection. It would also be of interest to gather information on sexual practices which was beyond the scope of this study.

In conclusion, we have identified hand carriage of genital HPV types in patients with genital warts. Although sexual intercourse is considered the usual mode of transmitting genital HPV infection, our findings raise the possibility of transmission by finger-genital contact.

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Contributors: C Sonnex supervised the collection of clinical samples and was the main author; S Strauss performed the polymerase chain reaction and was the author of the laboratory methods section; JJ Gray supervised the polymerase chain reaction and was co-author.

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