HPV typing of vulvovaginal condylomata in children

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Objective. To determine the human papillomavirus (HPV) subtypes in vulvovaginal warts in prepubescent children.

Design. Histopathology case series.

Setting. Outpatient and gynaecology clinics of hospitals in the greater Johannesburg area.

Patients. All cases of vulvovaginal warts diagnosed in children under the age of 12 years received at the South African Institute for Medical Research, Johannesburg, during the period 1 January 1991 to 31 December 1993.

Main outcome measures. Positivity for 'genital' HPV types 6, 11, 16, 18, 31, 33 and 35 using non-isotopic *in situ* hybridisation (NISH) and polymerase chain reaction (PCR).

Results. Eight of the 9 vulvovaginal warts contained HPV 11 when assessed by means of NISH (89%). PCR amplified HPV DNA in all 9 (100%) of the biopsies.

Conclusion. Detection of genital subtypes of HPV in childhood condylomata acuminata points strongly to sexual abuse, but should only be used as a guide to further investigation by a multidisciplinary team.

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Condylomata acuminata (CA) are anogenital warts caused by human papillomaviruses (HPVs) and are usually sexually transmitted.[1] Athough more than 70 HPV types have been identified, they are to a large extent body-site specific.[2,3] The subtypes most commonly associated with CA are 6, 11, 16 and 18.[4] Respiratory papillomatosis in young children is also associated with HPV 6 and 11.[1,4] HPV type 1 causes most plantar warts while HPV 2 is responsible for most common warts.[1]

This retrospective study was undertaken to: (*i*) determine the incidence of 'genital' HPV types in vulvovaginal warts in prepubescent children; (*ii*) identify those children in whom possible sexual transmission had occurred; (*iii*) identify anogenital lesions with 'oncogenic' potential in childhood. The latter is significant as the incidence of genital cancer in South African women is high.[5]

Subjects and methods

Vulvovaginal warts diagnosed in this department in children under the age of 12 years between January 1991 and December 1993 were reviewed.

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Paraffin wax-embedded blocks and haematoxylin and eosin-stained slides from 9 children were retrieved from the archive files. All patients were black girls aged between 3 and 12 years. Five of the condylomata were vulval in origin, while the remaining 4 were removed from the vagina (Table I).

Table I. Summary of clinical details (age and site of lesion), HPV typing with NISH and PCR confirmation of the presence of the virus

Case No.	Age (yrs)	Site	NISH HPV type	PCR
1	< 12*	Vulva	11	+
2	9	Vagina	—	+
3	12	Vagina	11	+
4	4	Vagina	11	+
5	6	Vagina	11	+
6	12	Vulva	11	+
7	10	Vulva	11	+
8	8	Vulva	11	+
9	6	Vulva	11	+

*This child was in a paediatric ward.

As this was a retrospective study, information on the presence of non-genital warts in these children and close family members was not available.

Non-isotopic in situ *hybridisation (NISH)*

NISH was performed on all 9 biopsy specimens with digoxigenin-labelled probes for HPV 6, 11, 16, 31 and 33 (Kreatech Biotechnology, Amsterdam). The methodology followed similar procedures used previously for HPV detection in cervical neoplasia,[6] the only exception being the use of diaminobenzidene (Sigma, SA) for the final detection step. (Chemicals used in this study were obtained from Boehringer, Merck, Dako and Sigma.) HPV 16- and 33-positive control cervical biopsies were included in each experiment.

Polymerase chain reaction (PCR)

PCR on paraffin wax sections was conducted on all biopsies as previously described.[7] HPV sequences from the E6 gene were amplified from paraffin sections with degenerate consensus primers to produce a product of 240bp. This E6 amplification system is highly specific but has a somewhat narrower HPV type spectrum. Human β-globin was used as an internal control amplification system to produce a 268bp product. Both systems were amplified independently; PCR products were viewed separately on ethidium bromide-stained agarose gel electrophoresis with an appropriate molecular marker to avoid misinterpretation. HPV 16- and 33-positive cervical biopsies (paraffin sections) were used as positive controls. In HPV-negative controls, tissue digests were omitted, and replaced by sterile water. The PCR and ISH analyses were carried out blind.

Results

Histology

Review of the histological findings confirmed the classic morphological features of CA: acanthosis, papillomatosis and parakeratosis. The superficial and intermediate keratinocytes showed prominent koilocytosis, while basal cell hyperplasia was present (Fig. 1).

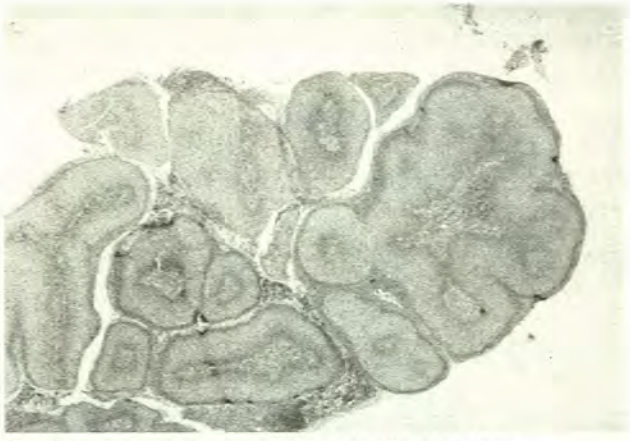

Fig. 1. Low-power microscopic section of a condyloma accuminatum showing classic morphological features.

The dermis showed a predominantly perivascular chronic inflammatory infiltrate. No dysplasia or malignancy was evident in any of the biopsies.

NISH

Eight of the 9 vulvovaginal warts contained HPV-11 with NISH (89%) (Fig. 2). A weak signal was also present on sections probed with HPV 6. This is not an unexpected finding as the sequence homology of these two HPV types is > 90%.

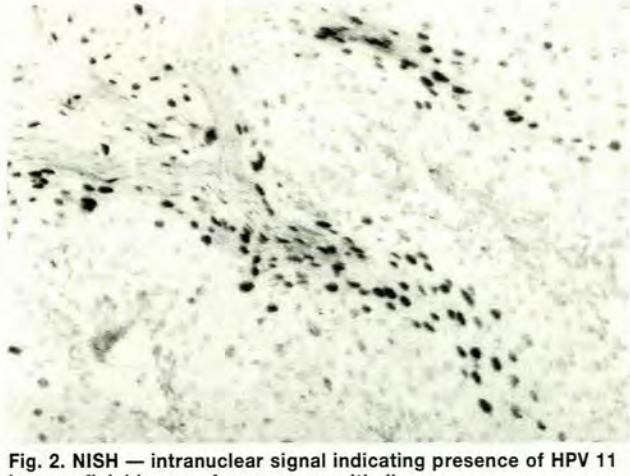

Fig. 2. NISH — intranuclear signal indicating presence of HPV 11 in superficial layers of squamous epithelium.

The remaining HPV types probed by means of NISH were negative. The distribution and location of HPV DNA was within the upper two-thirds of the epithelium, specifically within the areas showing morphological evidence of wart virus change. A diffuse (type 1) intranuclear signal was evident in all cases.[6]

PCR

PCR analysis was carried out blind on all 9 anogenital warts. This was used as an adjunct to NISH and to assess the efficacy and reliability of NISH (Table I). Human β-globin was amplified (268 bp band on gel electrophoresis) in 9 biopsies including the normal biopsy. HPV DNA was amplified (240 bp band on gel electrophoresis) in 9/9 (100%) of the

remaining biopsies (Fig. 3). HPV DNA and β-globin were amplified in both HPV 16- and 33-positive controls.

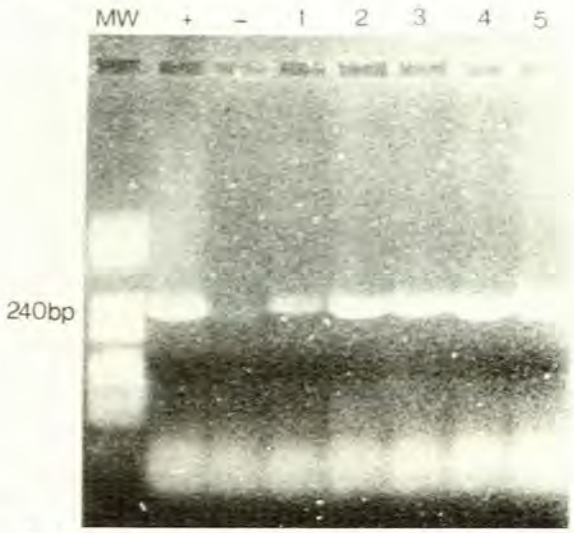

Fig. 3. PCR — amplified HPV DNA (240 bp band on gel electrophoresis) from 5 vulvovaginal wart biopsies. The positive control was a HPV 16-positive cervical biopsy and the negative control had tissue digest replaced with water (PBR 322 Hae III digest was used as a molecular marker) (MW = molecular weight marker; + = positive control; — = negative control; 1 - 5 = samples).

Negative controls comprising water instead of tissue digest did not show any amplification bands on gel electrophoresis.

Discussion

The incidence of anogenital warts in both adults and children is increasing.[3,8] This is of concern, especially in view of the increased awareness of sexual abuse of children and the long-term neoplastic potential of these lesions. Although CA in adults are regarded as sexually transmitted, a number of modes of transmission of HPV virus to the genitalia of children have been proposed.

1. **Vertical transmission** from the infected maternal genital tract during labour, particularly in children under the age of 2 years.[2,9] Occasional reports of infants born with congenital warts suggest possible *in utero* transmission of the virus.[3,10] Respiratory papillomatosis in children is associated with 'genital' HPV types 6 and 11, acquired through passage of the fetus through an infected maternal birth canal.[1,4]

2. **Auto-inoculation of non-genital warts** to the child's own genitalia is another possibility. HPV-typing, however, should reveal HPV types 1 to 3. In addition, these warts are usually of the verruca vulgaris type. However, it should be remembered that a skin type HPV genome may be associated with digital sexual abuse. Although this study did not probe for these cutaneous HPV types, 89% of our cases were positive for 'genital' HPV types with NISH.

3. **Possible direct non-sexual contact** with an HPV-infected family member, or indirect contact with HPV-infected fomites such as towels.[3]

4. **Sexual transmission** continues to elicit many controversial opinions. Some authors believe that in children older than 1 year, CA must be considered a sexually transmitted disease,[11] while others believe that 40 - 80% of cases are attributable to non-sexual encounters.[12] The necessity for HPV typing of condylomata in children is also hotly debated. The majority of all genital warts in children are caused by HPV 6 and 11,[13] as confirmed in the present study. The confirmation of 'genital' HPV types in anogenital warts in children, although not conclusive proof of sexual transmission, warrant careful investigation for sexual abuse.[1,13,14]

A careful multidisciplinary approach to the investigation and management of anogenital warts is advocated, encompassing paediatric, dermatological, gynaecological and community services.[14,15]

1. A correct histopathological diagnosis must first be made as skin tags, naevi and neurofibromas may mimic CA. It is therefore imperative that all anogenital warts be excised and submitted for histopathological examination and HPV typing. Definite documentation of the nature of these lesions is also necessary in those cases of sexual abuse which may require legal intervention.

2. A complete physical examination should be performed; any non-genital warts and any evidence of sexual abuse, such as injuries or bruises to the genitalia, should be documented.

3. Appropriate microscopic and serological studies for STDs (including HIV) should be undertaken; samples should be taken from areas such as the anopharynx, genitals and rectum.

4. Psychological assessment of behaviour, psychosocial and social signs by trained personnel is recommended.

5. Screening of adult family members for non-genital and genital warts and other anogenital infections should ideally be performed, although compliance may prove problematic.

6. Treatment of the lesions in children depends on the location, severity and type of wart, as well as the age of the child. If possible, excision of the lesion to allow HPV subtyping is recommended.

7. If 'oncogenic' HPV subtypes 16, 18, 31, 33 or 35 are detected in the lesions excised, careful long-term follow-up of these children is mandatory for the early detection of dysplastic or neoplastic anogenital lesions.[13]

Although the presence of genital subtypes of HPV in childhood CA points strongly to sexual abuse, careful investigation of each case is advocated to prevent both unnecessary trauma to the child and unfair incrimination of parents/relatives who may themselves require therapy for anogenital warts and screening for cervical neoplasia.

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