Plasmapheresis combined with immunosuppressive therapy (although expensive, time-consuming and likely to cause maternal morbidity) or high-dose intravenous immunoglobulin, are currently the only forms of therapy available to reduce very high antibody levels early in pregnancy at a stage when IUlVFs are technically very difficult to perform.

Babies who received IUlVFs should also be followed up for several months to monitor their haemoglobin levels and, if indicated, they should receive top-up transfusions. The place of erythropoietin in the management of this peculiar anaemia should be established to reduce the risk of transfusion-transmitted infections in the babies.

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REFERENCES


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HPV typing of vulvovaginal condylomata in children

C. A. Wright, L. Taylor, K. Cooper

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 8 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 genitinal warts caused by human papillomviruses (HPVs) and are usually sexually transmitted.1 Although 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. Respiratory papillomatosis in young children is also associated with HPV 6 and 11.4 HPV type 1 causes most plantar warts while HPV 2 is responsible for most common warts.5

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; and (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.6

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.
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

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Site</th>
<th>NISH HPV type</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 12</td>
<td>Vulva</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Vagina</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Vagina</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Vagina</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>Vagina</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>Vulva</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Vagina</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Vulva</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>Vulva</td>
<td>11</td>
<td>+</td>
</tr>
</tbody>
</table>

*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 Biotechnologies, Amsterdam). The methodology followed similar procedures used previously for HPV detection in cervical neoplasia, the only exception being the use of diamino benzidine (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. HPV sequences from the E6 gene were amplified from paraffin sections with degenerate consensus primers to produce a product of 240pb. 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 268pb 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).

Polymerase chain reaction (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 (268bp 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 warts.

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%.

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

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

Fig. 2. NISH — intranuclear signal indicating presence of HPV 11 in superficial layers of squamous epithelium.
remaining biopsies (Fig. 3). HPV DNA and ß-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.14 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.26 Occasional reports of infants born with congenital warts suggest possible in utero transmission of the virus.28 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.14

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 present in genital warts in children, although not conclusive proof of sexual abuse. Although the presence of genital subtypes of HPV in congenital warts suggest possible in utero transmission of the virus, 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, 99% 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.2

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,1 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\(^3\),12

A careful multidisciplinary approach to the investigation and management of anogenital warts is advocated, encompassing paediatric, dermatological, gynaecological and community services.13

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, genitai 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.

This study was supported by grants awarded to Professor K. Cooper from the South African Institute for Medical Research, the National Cancer Association and the Medical Research Council. The authors are grateful to Mrs N. Coetzee for her secretarial assistance.

REFERENCES

Hormones and growth factors in breast cancer

W. R. Bezwoda, L. Seymour, R. Dansey, D. Dajee, N. Mansoor

Hormonal treatment of cancer began in 1896 when Beatson described the use of oophorectomy for treatment of inoperable breast cancer. Abblative endocrine therapy, removing sources of oestrogenic hormones, either by oophorectomy or by ablation of extra-ovarian sites of oestrogenic hormone production, became the major therapeutic modality for patients with advanced breast cancer. These studies led to the theory that certain hormones were somehow necessary for the maintenance and growth of specific cancers.

Table I. Endocrine treatment for advanced breast cancer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Response rate (%)</th>
<th>Response duration (mths)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oophorectomy</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>33</td>
<td>25</td>
</tr>
<tr>
<td>Additive therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Oestrogens</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Progestogens</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>

Additive hormonal treatment (Table I) became available during the 1950s with the commercial production of steroid hormones. Since involution of the embryological breast bud which develops in all early, sexually undifferentiated embryos was known to occur under the influence of androgens it was perhaps not surprising that androgens could cause regression of female breast cancer. More surprising perhaps was the finding that oestrogens, which appeared to be necessary for the maintenance and growth of breast cancer in younger women, could also be beneficial, albeit usually in older, postmenopausal women. Other steroid hormones such as the gestagens and modified androgens such as danazol are also active.

The term 'changing the hormonal milieu' was coined to indicate a hypothetical alteration between growth-stimulatory and as yet (undefined) growth-inhibitory hormonal influences.

During the 1960s much effort went into attempts to define levels of plasma hormones or of total hormone production as measured by urinary excretion of sex steroids; this was intended to provide an index of hormone responsiveness and prognosis in breast cancer. Ultimately none of these tests achieved any real measure of success since these investigations focused on patient determinants rather than the nature of the tumour. However, several clinical features were defined which provided some guidelines for hormone treatment of breast cancer (Table I). In the absence of more specific information, these clinical guidelines remain useful.

Table II. Clinical features and response to hormone treatment in advanced breast cancer

<table>
<thead>
<tr>
<th>Feature</th>
<th>Favourable</th>
<th>Unfavourable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&gt; 60</td>
<td>&lt; 45</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Premenopausal</td>
<td>Rapid development of metastases</td>
</tr>
<tr>
<td>Disease-free interval</td>
<td>Bone, skin, node</td>
<td>Visceral metastases</td>
</tr>
</tbody>
</table>

Hormone receptors and the response of breast cancer to hormonal manipulation

Of course breast cancer is not the only tissue responsive to oestrogens. Normal breast and uterine tissue responds to oestrogens while at the same time oestrogenic hormones have no apparent effect on the growth of other organs. Early studies using both animal and human models helped to define oestrogen-responsive tissues and to demonstrate the association of a targeting mechanism with specific accumulation against a concentration gradient of oestrogen in responsive tissues. The first successful methods of measuring potential hormone responsiveness were based on the phenomenon of binding of oestrogen, and involved the use of a specific cellular receptor with high affinity for oestrogen that was able to recognise and competitively bind it. A specific oestrogen receptor (ER) was subsequently found in oestrogen-responsive tissues and in a proportion of breast tumours that were excised and kept fresh enough for the phenomenon to be demonstrated by a number of 'ligand-binding' methods.