Comparative in situ hybridisation study of juvenile laryngeal papillomatosis in Papua New Guinea and Australia

R G Wright, D P Murthy, A C Gupta, N Cox, R A Cooke

Abstract
A comparative study of cases of juvenile laryngeal papillomatosis from Papua New Guinea (n = 3) and Brisbane, Australia (n = 9) was carried out. In situ hybridisation reactions for human papillomavirus (HPV) types 6 and 11 occurred in 11 cases. All three cases from Papua New Guinea and eight from Australia gave positive signals. A negative reaction was observed in one Australian case. The intensity of the reaction was strong in seven cases, moderate in one, and weak in three. An equivocal reaction was also noted with probes for types 16 and 18, and types 31, 33, and 35 in two cases from Australia and one from Papua New Guinea.

It is concluded that as similar staining patterns and intensities occurred in cases from both areas, the aetiology is the same. The equivocal reactions noted in three cases were probably due to cross hybridisation rather than multiple infection.

Juvenile laryngeal papillomatosis is part of a spectrum of diseases characterised by recurrent papillomata of the mucous membrane of the upper respiratory and food passages. The larynx is the site most often affected, and is often the only site. Lesions are frequently multiple and often recur even after radical treatment.

The condition is manifest clinically with progressive huskiness of the voice. Stridor and acute respiratory obstruction may occur late in the disease, necessitating tracheostomy. The incidence of papillomatosis is low, but its management is difficult because of its tendency for rapid recurrence.

A viral aetiology for laryngeal papillomatosis was first suggested in 1928. Viral particles in the specimens have been shown electron microscopically. The presence of human papillomavirus (HPV) has been shown by immunofluorescence techniques, and HPV types 6 and 11 have been shown in papillomata by Southern blotting techniques with radioactive probes.

In situ hybridisation with radioactive probes permits the identification and localisation in tissue of HPV subtypes. Recently this has also been accomplished with biotinylated probes, a rapid method suitable for routine laboratory use. A recent large series of adult and juvenile cases showed positive reactions for HPV types 6 and 11 in about two thirds of the cases studied. Another series of juvenile cases, however, showed 100% positivity for HPV types 6 and 11.

Juvenile laryngeal papillomatosis has been well documented in Papua New Guinea and in Australia. This study was carried out to evaluate in situ hybridisation reactions and to compare the prevalence of HPV types 6 and 11 in cases of juvenile laryngeal papillomatosis from both regions.

Methods
Paraffin wax embedded tissue from 12 cases diagnosed in Papua New Guinea (n = 3) and Brisbane, Australia (n = 9) was retrieved from the pathology files of the Department of Pathology, Faculty of Medicine, Port Moresby General Hospital, Papua New Guinea and the Department of Anatomical Pathology, Royal Brisbane Hospital. The clinical features of the 12 cases were sought and these are presented in table 1. Most of these patients were female. Hoarseness of voice was the most common symptom. Multiple recurrent lesions and lesions at multiple sites were commonly observed.

Paraffin wax sections from formalin fixed tissues were cut at 5 μm and placed on slides pretreated with 3-aminopropyltriethoxysilane. The in situ hybridisation was carried out using the HPV tissue hybridisation kit (Vira Type in situ; Life Technologies, Inc—Bethesda Research laboratories, Gaithersburg, Maryland, USA). Biotinylated probes for HPV genotypes 6 and 11, 16 and 18 and 31, 33, and 35 were used, with one section for each probe group.

The method entails partial denaturation of DNA (heating the sections at 100°C on metal trays in an oven following proteolytic digestion and application of the probes), incubation with hybridisation mixture (at 37°C for two hours), detection of hybridisation using a streptavidin alkaline phosphatase conjugate which binds to the biotin on the probe and dephosphorylation by alkaline phosphatase of the substrate 5-bromo-4-chloro-3 indolyl-phosphate (BCIP) in the presence of nitroblue tetrazolium (NBT). The reaction results in deposition of purplish blue precipitates at the sites of probe hybridisation to HPV DNA. The method described is based on...
Table 1  Cases of juvenile laryngeal papilloma studied by in situ hybridisation

<table>
<thead>
<tr>
<th>Case No</th>
<th>At first presentation</th>
<th>Sex</th>
<th>Country</th>
<th>Clinical features at first presentation</th>
<th>Site of papilloma</th>
<th>Treatment</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 y 6 m</td>
<td>F</td>
<td>Papua New Guinea</td>
<td>Progressive hoarseness of voice and dyspnoea—3 months</td>
<td>Uvula, right tonsillar pillar; tip of epiglottis, true and false vocal cords, anterior commissure; cricoaryte</td>
<td>Initial tracheostomy. Subsequent excision with cupped forceps and microlaryngoscopy</td>
<td>15 months; multiple recurrences</td>
</tr>
<tr>
<td>2</td>
<td>5 y</td>
<td>F</td>
<td>Papua New Guinea</td>
<td>Inability to talk clearly and respiratory stridor—5 months</td>
<td>False and true vocal cords</td>
<td>Excision with cupped forceps</td>
<td>3 m</td>
</tr>
<tr>
<td>3</td>
<td>1 y 7 m</td>
<td>M</td>
<td>Papua New Guinea</td>
<td>Progressively increasing dyspnoea 6 months</td>
<td>True and false vocal cords; anterior commissures, Trachea</td>
<td>Initial tracheostomy subsequent excision with forceps</td>
<td>6 m</td>
</tr>
<tr>
<td>4</td>
<td>7 y 7 m</td>
<td>F</td>
<td>Australia</td>
<td>Increasing respiratory distress, huskiness of voice—6 months</td>
<td>Vocal cords, epiglottis, pharynx</td>
<td>Initial tracheostomy followed later by excision with forceps</td>
<td>3 y</td>
</tr>
<tr>
<td>5</td>
<td>9 y 6 m</td>
<td>F</td>
<td>Australia</td>
<td>Hoarseness of voice; recurrent laryngeal papilloma since the age of 7 y</td>
<td>Vocal cords, anterior commissure, laryngeal surface of epiglottis</td>
<td>Diathermy excision</td>
<td>9 y 11 m</td>
</tr>
<tr>
<td>6</td>
<td>9 m</td>
<td>F</td>
<td>Australia</td>
<td>Difficulty in breathing—3 weeks, stridor; distress</td>
<td>True and false vocal cords</td>
<td>Diathermy excision</td>
<td>1 y 8 m</td>
</tr>
<tr>
<td>7</td>
<td>2 y</td>
<td>F</td>
<td>Australia</td>
<td>Vomiting, respiratory stridor, hoarseness of voice 6 months, noisy breathing and difficulty—since the age of 5 months</td>
<td>Right and left vocal cords, posterior pharyngeal wall in hypopharynx</td>
<td>Multiples excisions</td>
<td>5 m</td>
</tr>
<tr>
<td>8</td>
<td>4 y 6 m</td>
<td>F</td>
<td>Australia</td>
<td>Hoarseness of voice</td>
<td>False cords (right and left)</td>
<td>Multiple excisions</td>
<td>1 y 7 m</td>
</tr>
<tr>
<td>9</td>
<td>1 y 6 m</td>
<td>F</td>
<td>Australia</td>
<td>Huskiness of voice—1 month</td>
<td>Vocal cords (right and left) Inferior surface of epiglottis</td>
<td>Multiple excisions</td>
<td>3 y 5 m</td>
</tr>
<tr>
<td>10</td>
<td>4 y 4 m</td>
<td>F</td>
<td>Australia</td>
<td>Hoarseness of voice</td>
<td>Both vocal cords, posterior pharyngeal wall, posterior oesophageal wall</td>
<td>Multiple excisions</td>
<td>1 y 2 m</td>
</tr>
<tr>
<td>11</td>
<td>11 y 8 m</td>
<td>M</td>
<td>Australia</td>
<td>Gradual onset of hoarseness of voice</td>
<td>Both vocal cords</td>
<td>Excision</td>
<td>5 y</td>
</tr>
<tr>
<td>12</td>
<td>6 y 6 m</td>
<td>M</td>
<td>Australia</td>
<td>Hoarseness of voice—2 years</td>
<td>Vocal cords, pharyngeal surface of arytenoid, anterior commissure</td>
<td>Excision</td>
<td>1 y 7 m</td>
</tr>
</tbody>
</table>

Results

The reaction with the probe for HPV types 6 and 11 was found in 11 of the 12 cases studied (table 2). All three cases from Papua New Guinea and eight of the nine Australian cases gave positive signals. A negative reaction was observed in one Australian case. The reaction with the probe for HPV types 6 and 11 was present in the nuclei of the superficial epithelium in the positive cases (figure). No difference in the pattern of reaction of the probes was observed between the two groups of cases from the two regions. The intensity of the reaction was strong in seven cases, moderate in one case, and weak in three. An equivocal reaction was also noted with probes for types 16 and 18, and types 31, 33 and 35 in three cases, two from Australia and one from Papua New Guinea.

Discussion

The results of the in situ hybridisation tests for HPV types 6 and 11 show similar patterns and intensity of staining in cases from both areas. The results obtained are in keeping with those reported from other areas. Quiney has speculated that variation in hybridisation reactions may be the result of variation in fixation of tissue rather than...
be due to this cross reactivity rather than to the presence of a multiple infection. We consider that caution in the interpretation of these reactions should prevail and support the methods of interpretation outlined by Quiney et al.
