DETECTION OF HUMAN PAPILLOMAVIRUS L1-16 AND -18 DNA AND EPSTEIN-BARR VIRUS DNA IN LARYNGEAL CARCINOMA

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CLC number: R739.6 Document code: A Article ID: 1000-9604(2005)-02-0120-06

ABSTRACT

Objective: To look for the further evidence for HPV L1 HPV16 E6, HPV 18 E6 and EBV as carcinogenic factors in laryngeal carcinoma. Method: we examined representative numbers of specimens from laryngeal cancer with highly sensitive PCR technique for the presence of HPV L1 and high-risk types HPV16 E6, HPV 18 E6 and EBV LMP1. Results: Using PCR detection, 7.3% samples were HPV L1 positive, 52.03% were HPV16 E6 positive, 30.89% were HPV18 E6 positive and 9.13% were EBV LMP1 positive. The low incidence of HPV L1 and high incidence of HPV-16 E6 and HPV18 E6 genes suggest that HPV might be integrated into tumor cells. Our results support a role of HPV-16 and HPV-18 infection in the pathogenesis of laryngeal carcinoma in China. Conclusion: Integration of E6 into host genome and stable expression of these genes may be associated with the carcinogenesis of laryngeal carcinoma. HPV-16 and HPV-18 may synergistically function on the pathogenesis of laryngeal carcinoma. Our results suggest an association of laryngeal carcinogenesis and infection with the high-risk HPV types 16, HPV 18 and EBV.

Key words: EBV; HPV; Polymerase chain reaction; Laryngeal carcinoma

The aetiology of laryngeal cancer has yet to be clarified, despite its importance among head and neck malignancies. Laryngeal cancer accounts for almost 1.2% of all cancers in USA[1]. In Spain, france and Italy the incidence of laryngeal cancer is 10/100000/year, greater than in the U.K. and China where it is 4/100000/year[2].Almost all laryngeal cancers(90-95%) are squamous cell carcinoma (SCC). Generally, the peak incidence is in the sixth and seventh decades of life[3], with a clearcut male preponderance, although this may change in the future due to increased smoking habits

Received date: April 15, 2005; Accepted date: May 27, 2005.
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distributed in human population. Mainly transferring of saliva between individuals spreads primary infection of EBV. Following primary infection, either symptomatic or silent, the virus persists in the host throughout the entire life, showing tropism for B lymphocytes, but is also able to infect T lymphocytes, follicular dendritic reticulum cells and less frequently epithelial cells[9]. EBV is the most common cause of infectious mononucleosis and also is associated with several human malignancies including Burkitt’s lymphoma, Hodgkin’s disease, nasopharyngeal carcinoma. A possible role of EBV in pathogenesis of breast cancer and gastric carcinoma has been discussed[10-13]. In this study we employed the PCR technique to detect Epstein-Barr virus (EBV) and human papillomavirus (HPV) DNA in paraffin-embedded tissues from China patients with laryngeal carcinoma.

**MATERIALS AND METHODS**

**Patients and Laryngeal Carcinoma Tissue Specimens**

From December 1995 to March 2005, A total of 132 patients of laryngeal cancer ranging age from 38 to 75 years (mean 57.32) including 98 men and 34 women were included in our study. All cases of formalin fixed paraffin embedded laryngeal carcinoma tissue specimens were collected from Beijing Cancer Hospital and Shougang Hospital. All cases were diagnosed as laryngeal carcinoma by histological analysis.

Histological analysis identified 65 patients with well differentiated, 49 with moderately differentiated and 18 with low differentiated squamous cell carcinoma (SCC).

**DNA extraction**

DNA extracts were prepared from five sequential paraffin sections from laryngeal carcinoma specimens, using a method developed in our laboratory. Briefly, DNA was extracted from paraffin-embedded tissues collected in 1.5-ml Eppendorf tubes. A 400ulmquantity of digestion buffer containing 100 g/ml proteinase K were added to each tube. After 1 h of incubation at 60°C, fresh proteinase K was added at a concentration of 100g/ml and incubation was continued for 1 h more. Samples were then extracted with equal volumes of phenol, phenol/chloroform and chloroform. DNA was precipitated with the addition of 20 ul 5 N NaC1 and 1 ml ethanol. Eppendorfs were stored at -20°C overnight. DNA was recovered by centrifugation at 13000 rev/min for 15 min at 4°C, washed with 70% ethanol and the pellet was resuspended in 20-50 ul distilled water.

**Oligonucleotide Primers**

To detect EBV and HPV DNA, we used primers specific for the LMP1 region of the EBV genome and HPV L1, HPV16 E6 and HPV18 E6. Primers flanking the b-globin gene were used as controls to make sure that adequate amounts of DNA were present in the samples during isolation from the tissue. Their sequence and position on the genomes are indicated in Table 1.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV L1</td>
<td>F: GCACAGGG(TC)CA(CT)AA(CT)AATGG</td>
<td>450bp</td>
</tr>
<tr>
<td></td>
<td>R: CTTG(TG)ACTA(AG)AGGA(CT)TGATC</td>
<td></td>
</tr>
<tr>
<td>HPV16E6</td>
<td>F: GCAAGCAACAGTTACTGCGA</td>
<td>321bp</td>
</tr>
<tr>
<td></td>
<td>R: CAACAAGACATACATCGACC</td>
<td></td>
</tr>
<tr>
<td>HPV18E6</td>
<td>F: GCGCTTTGGAGATCCAACAC</td>
<td>415bp</td>
</tr>
<tr>
<td></td>
<td>R: ACGAATGGCAGCTGCTC</td>
<td></td>
</tr>
<tr>
<td>EBV LMP1</td>
<td>F: TCATCTCGTCTAATTGCTC</td>
<td>145bp</td>
</tr>
<tr>
<td></td>
<td>R: TCA(TC)CTGCTCATTGCTC</td>
<td></td>
</tr>
</tbody>
</table>

**PCR Conditions**

The PCR reaction was performed using 100 ng of tissue extracts in a final volume of 50 ml. The PCR mixture contains 2.5 units of Thermus Aquaticus (Taq) DNA polymerase, 5 ml of 10x reaction buffer, 3 ml of 25 Mm MgCl2, 1 ml of 2.5 mM of each deoxynucleotide triphosphate, and 250 ng of each primer. The PCR was performed out using a PTC-100-60 programmable thermal controller (MJ Research Watertown, MA). The reaction mixture was amplified for 35 cycles at an initial denaturation step at 95°C for 5 min in the Thermal