Study on the association between squamous cell carcinoma of larynx and HPV by PCR

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Abstract

Introduction: Abroad epidemiologic studies have suggested human papillomavirus (HPV) to be an etiological agent in Squamous cell carcinoma (SCC) of larynx. In this study we investigated the frequency of HPV 16 infection in various neoplastic and non-neoplastic laryngeal tissue.

Materials and Methods: The study was done on 55 cases of laryngeal SCC, 10 cases of dysplastic and 5 cases of normal tissues were analyzed by polymerase chain reaction (PCR) for the presence of HPV 16.

Results: None of the five normal and ten dysplastic laryngeal tissues showed the presence of HPV. Seven of fifty-five Squamous cell carcinoma of larynx (12.7\%) were positive for HPV type 16.

Conclusions: The results suggested that HPV DNA has a major role in squamous cell carcinoma of larynx

Keywords: HPV, Squamous cell carcinoma, Larynx

Introduction

Laryngeal Squamous Cell Carcinoma (SCC) is the most frequent malignant tumor in the head and neck. Human Papilloma Virus (HPV) has been implicated in proliferative and invasive lesions of the larynx (1-9). More than 75 different HPV genotypes have been cloned, and categorized in low and high risk types. The low risk types are associated with benign lesions and high risk HPV genotypes have marked malignant potential and are frequently associated with epithelial dysplasia and Squamous Cell Carcinoma at several anatomic sites.

The low risk HPV-6 and 11 DNA sequences were detected in approximately 100\% of Laryngeal Papilomatosis (10) and the high risk HPV (16,18) have been found in high percentage of Laryngeal Verrucous Carcinoma (11).

Although almost all epithelial cancers of head and neck and genital system harbour HPV DNA and the relationship between HPV and anogenital cancers have been clearly demonstrated in numerous studies, a putative relationship between HPV and Laryngeal Carcinoma is still controversial.
In 1986 HPV DNA was cloned from a laryngeal carcinoma (1) and HPV DNA has been demonstrated in carcinomas arising from preexisting laryngeal papilloma (2,4). Although epidemiologic investigations have unquestionably established tobacco and alcohol as major risk factors for Squamous Cell Carcinoma of the larynx (2), the role of human Papilloma virus (HPV) in this tumor is still unclear. HPV is double stranded DNA virus with a circular genome of 7600-8000 base pairs that replicate only within the permissive host cells (3).

The life cycle of HPV is tightly linked to squamous cell differentiation that cause abnormal maturation and differentiation of epithelial cell (4). Hence in the present study, we analyzed HPV infection in neoplastic and non-neoplastic laryngeal tissues.

**Materials and Methods**

55 samples of invasive squamous cell carcinoma and 10 sample of dysplastic and 5 samples of normal laryngeal tissues were included in this study. Specimens were fixed in 10% formalin, 10-15 μm thick sections taken from paraffin embedded tissue and stained with Hematoxylin-Eosin for routine histopathologic examination. Blocks without malignant tissue were omitted but all other specimens were selected with enough tumoral component. Sections for each block were cut for PCR. Control sections before and after sectioning for PCR were cut and stained with H&E in order to ensure that the sections for PCR did contain tumor tissue. The sections for PCR were placed in a 1.5 ml tube and 200 μl digestion buffer (10 mM Tris pH=7.0, 1mM EDTA, 200 μg/ml Proteinase K) was added. The sections were digested at 65°C for 10 min. The Samples together with appropriate positive and negative controls, were amplified with primers targeting a 315 bp fragment of the beta-actin gene in order to ensure the integrity of the DNA.

Finally PCR was performed with the universally accepted consensus primers HPF (GP65) and HPR (GP66) with some modification. The amplified samples were evaluated by gel electrophoresis and photographed under UV transillumination.

**Results**

PCR amplification of HPV DNA showed that all normal and dysplastic laryngeal tissue were negative for HPV and 7 of 55 cases Squamous cell carcinoma of larynx were positive for HPV16 (12.7%) Of the invasive carcinomas which examined for HPV, 29% were well differentiated, 41% were moderately differentiated, and 29% were poorly differentiated. There was no correlation between the site of the tumor and the detection of HPV. There was also no significant correlation between the presence of HPV in laryngeal carcinoma and clinicopathologic aspects such as age, site, grade, of the laryngeal carcinoma (table 1,2,3) (figure 1).

**Table 1: HPV positivity laryngeal tissue**

<table>
<thead>
<tr>
<th>Larynx</th>
<th>Number</th>
<th>HPV PCR Positive (%)</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>(0)</td>
</tr>
<tr>
<td>Dysphasia</td>
<td>10</td>
<td>(0)</td>
</tr>
<tr>
<td>SCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>23</td>
<td>(7.22)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. distribution of laryngeal SCC according to site**

<table>
<thead>
<tr>
<th>Site</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supraglottic SCC</td>
<td>31 (56.3)</td>
</tr>
<tr>
<td>Glottic SCC</td>
<td>16 (29)</td>
</tr>
<tr>
<td>Infraglottic SCC</td>
<td>8 (14.5)</td>
</tr>
</tbody>
</table>
Table 3. Laryngeal carcinoma (SCC) according to age and sex.

<table>
<thead>
<tr>
<th>Mean of age (years)</th>
<th>(range)</th>
<th>Male (%)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>37-82</td>
<td>43(28.1)</td>
<td>12(21.8)</td>
</tr>
</tbody>
</table>

Several Studies showed that high risk HPV 16-18 and 33 were positive in 51% of 102 laryngeal SCC in northeast of China. Some researchers suggested that the high detection ratio of HPV in laryngeal SCC resulted from contamination (19).

In this study, however every effort was made to avoid contamination. Probably the difference in the prevalence of HPV infection may partly depends on the primers used in individual studies. The biological activities between high risk and low risk HPV mainly due to the different binding affinity of the E6 and E7 proteins of the E6 and E7 proteins to the tumor suppressor proteins P53 and Rb. The possible pathogenic role of HPV in laryngeal carcinoma has also been studied (20).

He believed that HPV 16 was the most type that infected laryngeal carcinoma and other exogenous risk factors such as Nicotine and alcohol participate their actions. Several studies showed significant correlation between presence HPV and expression of PCNA (21-23).

This fact explains that high risk HPV can inactivate P53 leading loss of its normal anti proliferative activity (24). Other studies have shown that HPV can also directly activate cellular proliferation (25,26). A study on 102 laryngeal carcinoma from China showed P53 accumulation in 58% of HPV positive tumors (21).

Conclusion

Result of the present work agree with data from the literature that under scores a relevant prevalence of HPV sequence in larynx carcinoma. However questions remain open and might find answers in larger population-based studies.

Acknowledgments

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References

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خلاصه

مطالعه همبستگی بین سرطان سلول سگفشری حنجره و ویروس HPV

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مقدمه: بررسی های اپیدمیولوژیک نشان داده که این کازومهای انسانی (HPV) را در سرطان سلول سگفشری حنجره می‌تواند منجر به تشخیص بیماران در سطح بالای این نرمال حنجره بنماید. مورد تأیید قرار نمی‌دهد. در این مطالعه برای بررسی پرواژ ویروس پاپیلومای انسانی (HPV) و بیش از 16 و 17 نوع سلول سگفشری و بافت نرمال حنجره مورد بررسی قرار گرفت.

روش کار: این مطالعه در مورد 55 نمونه، 40 مورد کازوم سلول سگفشری حنجره و 10 مورد مخوف دیسپلازیک و 5 مورد بیش از 50 مورد کازوم سلول سگفشری حنجره جهت بررسی حضور PCR بناست و سلول حنجره با ویروس مولکولی PCR نتایج: وضعیت نرمال و 10 مورد دیسپلازیک حنجره هیچ کدام آگاهی در بین ویروس HPV را نشان ندادند. از 7 نمونه کازوم سلول سگفشری و 5 مورد حجم نرمال حنجره نکه گی: نتایج به دست آمده نشان می‌دهد که میزان DNA و ویروس پاپیلومای انسانی در کازوم سلول سگفشری حنجره نرمال حنجره.

می‌دهد.

واژه‌های کلیدی: ویروس پاپیلومای انسانی (HPV), کازوم سلول سگفشری حنجره.