No evidence of known types of human papillomavirus in squamous cell cancer of the oesophagus in a low-risk area
Kok, T.C.; Tjong-a-Hung, S.P.; Smits, H.L.; ter Schegget, J.

Published in:
European Journal of Cancer

DOI:
10.1016/S0959-8049(97)85984-9

Citation for published version (APA):
No Evidence of Known Types of Human Papillomavirus in Squamous Cell Cancer of the Oesophagus in a Low-Risk Area

for the Rotterdam Oesophageal Tumour Study Group, Rotterdam, The Netherlands

Controversial results regarding the presence and role of human papillomavirus in the development of oesophageal squamous cell carcinoma have been published. We used multiple broad-spectrum polymerase chain reactions to identify HPV DNA in oesophageal carcinomas from a low-incidence area. Paraffin embedded- and snap-frozen specimens from oesophageal cancer tissues of 63 patients were examined with a PCR technique with several primer pairs, capable of detecting most known HPV types. In none of the oesophagus cancer tissues could HPV DNA be detected. The role of HPV in this type of carcinoma in a low incidence area remains unclear.

Key words: oesophagus, neoplasm, human papillomavirus

INTRODUCTION

SQUAMOUS CELL carcinoma of the oesophagus is a highly lethal disease, with striking variation in incidence in different parts of the world. From epidemiological surveys, it has been suggested that excessive alcohol intake and use of tobacco (especially in combination), and possibly certain nutritional deficiencies (vitamins A, B and C) are some of the risk factors, but these factors alone cannot explain the very high incidence in some well-defined geographical areas in North China, Iran and South Africa.

Human papillomaviruses (HPV) have been found to play a causative role in the pathogenesis of cervical dysplasia and cervical carcinomas. In 1978, a possible interaction between a bovine papillomavirus (BP4) and an environmental carcinogen (bracken fern) was regarded as an important event for the development of squamous cell carcinomas, especially of the oesophagus, in cattle grazing on the Scottish Highlands [1]. Syrjäsen suggested in 1982 for the first time a possible aetiological relationship between HPV and benign proliferations of the squamous mucous membrane of the oesophagus, for instance, papillomas [2]. In the animal model of bovine papillomavirus infection, these lesions have been reported to undergo malignant transformation following exposure to carcinogens. Winkler and associates in 1985 reported the clinical, histological and morphological features of HPV infection in cases of benign oesophageal proliferations, while at the same time a role of HPV infection in carcinomas of the oesophagus in black South Africans was suggested [3,4]. In both studies, HPV antigens could be detected by means of immunoperoxidase techniques in 30% of those cases in which the histological criteria of HPV infection were met. Since then, a number of controversial studies have been published about the detection of HPV DNA in human oesophageal cancer with different techniques. Positive results were obtained mainly in high-incidence areas. In The Netherlands, squamous cell cancer of the oesophagus is a rare disease. We investigated the presence of HPV DNA in oesophageal cancer from a low-incidence area using multiple, very sensitive, broad-spectrum polymerase chain reaction (PCR) techniques.

MATERIALS AND METHODS

We investigated formalin-fixed, paraffin-embedded tumour specimens of 63 consecutive patients with operable invasive squamous or undifferentiated large cell carcinoma of the oesophagus, who participated in a phase III randomised clinical trial of surgery with \( n = 21 \) or without \( n = 42 \).
neoadjuvant chemotherapy. This study was carried out in the largest referral centre for oesophageal cancer patients in The Netherlands (age-adjusted death rate of oesophageal cancer: 7.9/100 000 for males and 3.2/100 000 for females). In 20 out of 42 patients treated with surgery alone, we also investigated snap-frozen specimens, collected and frozen in liquid nitrogen within 1 h after surgical removal. All available haematoyxlin and eosin stained sections were reviewed, and the most representative block was selected for further studies. Patient characteristics are listed in Table 1. No patient had a history of or a presence of active papillomas of any site in the head and neck or oesophageus region.

To extract DNA from paraffin-embedded tissue, 5 µm sections were cut, taking care to prevent cross-contamination, and incubated in 300 µl 10 mM Tris-HCl (pH 8.9), 50 mM KCl, 2.5 mM MgCl2 and 0.5% Tween-80 with 200 µg/ml proteinase K at 56°C for 18 h. At the end of the incubation, ylin and eosin stained sections were reviewed, and the most representative block was selected for further studies. Patient characteristics are listed in Table 1. No patient had a history of or a presence of active papillomas of any site in the head and neck or oesophageus region.

Table 1. Patient and tumour characteristics (n = 63)

<table>
<thead>
<tr>
<th>Median age</th>
<th>63.5 years (35–77 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>48</td>
</tr>
<tr>
<td>female</td>
<td>15</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>60</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
</tr>
<tr>
<td>Chinese</td>
<td>1</td>
</tr>
<tr>
<td>Grade of differentiation</td>
<td></td>
</tr>
<tr>
<td>well</td>
<td>7</td>
</tr>
<tr>
<td>moderately</td>
<td>38</td>
</tr>
<tr>
<td>poor</td>
<td>17</td>
</tr>
<tr>
<td>undifferentiated</td>
<td>1</td>
</tr>
<tr>
<td>Stage (UICC)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>II</td>
<td>26 (41%)</td>
</tr>
<tr>
<td>III</td>
<td>10 (16%)</td>
</tr>
<tr>
<td>IV</td>
<td>21 (33%)</td>
</tr>
</tbody>
</table>

RESULTS

None of the different PCR reactions resulted in the detection of HPV DNA in the oesophageal carcinoma specimens when the PCR products were analysed by ethidium bromide stained agarose gel, indicating that the sample was adequate for PCR analysis.

DISCUSSION

We investigated a possible role of HPV in the pathogenesis of squamous cell carcinoma of the oesophagus in patients from a low-incidence area. In this material, of which 95% of the specimens were proven to be suitable for PCR analysis, we could not detect any HPV DNA. These results strongly suggest that HPV DNA of the known HPV types is not present in at least the majority of the oesophageal squamous cell carcinomas in The Netherlands. We cannot exclude the possibility that novel HPV types, which do not match the PCR primer sets, are present. Recently, we succeeded in detecting novel HPV types in skin lesions of immunocompromised patients by PCR amplification, employing a nested PCR approach using the primer sets CP65/66 and CP69/70. In a separate reaction, a β-globin PCR was carried out using two pairs of primers; either PC03 (glo-1) and RS42 amplifying a 441 bp fragment or glo-1 (5'ACAACACTGT-GTTCACTACC) and glo-3 (5'TCTATTGGTCTCC-TAAAACC) amplifying a 172 bp fragment. Successful amplification of the β-globin fragment, visualised on an ethidium-stained agarose gel, indicates that almost all DNA preparations were adequate for PCR analysis.
in the nuclei of cancer cells by \textit{in situ} hybridisation, in 4% only in the adjacent epithelium with hyperplastic or dysplastic changes, and in only 1 case in the resected margin. HPV type 16 was the most common finding in the 85 positive cases [20]. The presence of positive hybridisation signals in regional lymph node metastases (12.3%), exclusively confined to the nuclei of metastatic cancer cells, suggests a causal association of HPV and oesophageal carcinoma in these patients, more so because the same viral type was invariably detected in both the primary tumour and the metastatic lesions. Cooper and associates demonstrated the presence of HPV DNA in 25 of 48 oesophageal cancers from a high-risk area (South Africa), utilising non-isotopic \textit{in situ} hybridisation with HPV DNA probes to HPV 6, 11, 16, 18, 31 and 33 [21].

Very recently, Dillner and associates found an association between seropositivity to HPV type 16 and the risk of oesophageal cancer in Finland [22].

In contrast to these data, negative results have also been reported, especially in low-risk areas, but also in high-risk areas [23-26]. Loke and associates applied both \textit{in situ} hybridisation and DNA slot blot analysis to a series of 37 cases where total oesophagectomy was performed for squamous cell carcinoma in the high-incidence area of Hong Kong. With both techniques no HPV was detectable in cancer cells nor in intra-epithelial neoplasia nor in normal oesophageal mucosa. Kiyabu and associates used \textit{in vitro} gene amplification by the polymerase chain reaction to look for HPV type 16 and 18 DNA in invasive squamous cell cancers of various types [23]. While 70% of the ano-genital carcinomas, and 36% of the oropharyngeal carcinomas contained HPV DNA sequences, none of 13 oesophageal carcinomas were found to be positive. Our own results from a low-incidence area are consistent with these studies.

There are several complicating issues when comparing studies about HPV detection in benign and malignant tissues. In high-risk areas, a possible influence of screening methods regarding the stage of the tumour at the time of diagnosis could exist. It is important to realise that several authors found IIHP DNA in the epithelium, adjacent to the carcinoma, more than in the cancer itself [16]. This phenomenon is consistent with the earlier findings that bovine papillomavirus type 4 DNA in high copy number could be readily identified in bovine papillomas, but no viral DNA nor viral antigens could be detected in malignant lesions, indicating that viral genomes are not necessary to maintain a malignant state.

A second issue is the methodology. Using amplification by the polymerase chain reaction, it is possible to detect HPV genomes with high sensitivity, for instance up to less than $10^{-1}$ copies of viral genome per cell, which is superior compared to the older hybridisation techniques and immuno-staining techniques [14, 15]. We used in our study several pairs of general primer sequences, which are conserved among a broad spectrum of HPV types, and which permit the detection of more HPV types in a single sample. Also potentially new HPV types can be identified by these methods.

In conclusion, in our study with oesophageal squamous cell cancer specimens from a low-incidence area, we could not confirm the results of some other investigators, identifying HPV DNA in oesophageal carcinomas, mainly type 16 and 18. In this respect, our results are identical to those described by Loke, Kiyabu, Sugimachi and Akutsu [22, 24–26].


