Evaluation of the role of human papillomavirus in oesophageal squamous cell carcinoma in Belgium

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Abstract

Objective : **To evaluate the putative role of human papillomavirus (HPV) in the aetiology of oesophageal squamous cell carcinoma (OSCC) in Belgium.**

Methods : **The frequency of HPV infection was determined using HPV DNA PCRamplification with L1 consensus primers MY09-MY11, able to recognise about 40 different HPV types, on twenty-one formalin-fixed and paraffin-embedded oesophageal squamous cells carcinomas. Nineteen samples of histologically normal epithelium from the surgical margins of the OSCC specimens and five samples from normal oesophagus obtained at autopsy served as negative controls.**

Results : **We found only one HPV positive tumour (4,8%) out of the 21 OSCC cases. All the normal epithelium controls remained negative.**

Conclusions : **Our data are in agreement with those previously published, suggesting that HPV infection only plays a minor role in the pathogenesis of oesophageal squamous cells carcinoma in West-European countries.** (Acta gastroenterol. belg., **2000**, 63, **154-156**).

Key words : squamous cell carcinoma, oesophageal carcinoma, polymerase chain reaction, human papillomavirus.

Introduction

Oesophageal squamous cell carcinoma is a neoplasm with very poor prognosis and striking geographic variation in incidence even within populations that presumably share a similar genetic background. The exact cause of the disease remains uncertain although many candidate risk factors have been exhaustively investigated during these last years. Cigarette smoking and heavy alcohol consumption, especially when associated, seem to be the leading risk factor in Western Europe and in North America. Other risk factors such as diet deficiencies in vitamins and proteins antioxidants, opium use and exposition to nitrosamines have been suggested to play a more important role m other countries with higher OSCC incidence (1).

Human papillomaviruses (HPV) have been widely studied these late years, using different molecular approaches. Their presence was detected in squamous cell carcinomas of several organs, mainly by PCR and in-situ hybridisation. However, the etiologic role of HPV infection has only been firmly established in the development of ano-genital squamous cell carcinomas (2).

The presence of HPV m some cases of OSCC was first suggested by Syrjänen (3) in 1982, and confirmed later on by various techniques as summarised by Poljak *et al.* in a recent review (4). However, its prevalence ranges from 0% to 66%, depending on the population tested and the detection technique used. So its role in OSCC carcinogenesis remains unclear, particularly in our country where, to our knowledge, it has not been previously studied.

Material and methods

The study group consisted in twenty-one patients who had undergone oesophagectomy for resection of primary squamous cell carcinoma at the Erasme Hospital. Alcoholic status and smoking habits from each patient were retrieved from the files. All pathological specimens were formalin-fixed paraffin-embedded material. Histologically normal mucosa from the surgical margins of the OSCC specimens was taken when available (19 cases). Five samples of normal mucosa coming from autopsy served as negative controls. Five C1N3 lesion of the uterine cervix previously proven to contain HPV 16 or 18 were used as positive controls.

The presence of HPV DNA was studied using the "hot start" polymerase chain reaction DNA amplification method on paraffin-embedded tissue as previously described, with L1 consensus primers MY11 (positive strand) and MY09 (negative strand) (5,6). These L1 primers allow to recognise more than 40 types of HPV including the most common mucosal oncogenic types (HPV type 16, 18, 31, 33, 35). All HPV previously described in association with OSCC could be easily detected by these primers. All precautions were taken to avoid cross-contamination (7). The DNA isolated from each tumour was amplified with Ll-consensus primers at low stringency conditions. PCR products were analysed on ethidium bromide-stained agarose gels.

The chi-square test was used to test for statistical difference between proportions of HPV DNA positive samples in normal mucosa and OSCC.

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Results

Viral DNA was correctly amplified in the five C1N3 lesions. None of the normal epithelium samples were positive for HPV DNA. The 268 bp-band of ß-globine was present in all negative samples, indicating the absence of possible PCR inhibitors.

Only one case of OSCC (4,8%) exhibited the expected 450 base-pair (bp) band consistent with HPV infection (Fig. 1). The HPV-positive sample originated from a rapidly progressive lesion graded pT3 N0 according to the UICC, appearing in a 64 year old man with no recorded history of warts or genital condylomas.

The difference between normal mucosa and OSCC samples regarding the presence of HPV DNA did not reach statistical significance ($p > 0,01$).

Discussion

HPV DNA has been detected in OSCC world-wide with a frequency ranging from 0 to 66% of the samples (4) (Fig. 2). The use of various detection methods such as morphology, immunohistochemistry, in-situ hybridisation, southern blot and PCR, may partly explain the wide variations of HPV prevalence observed in OSCC.

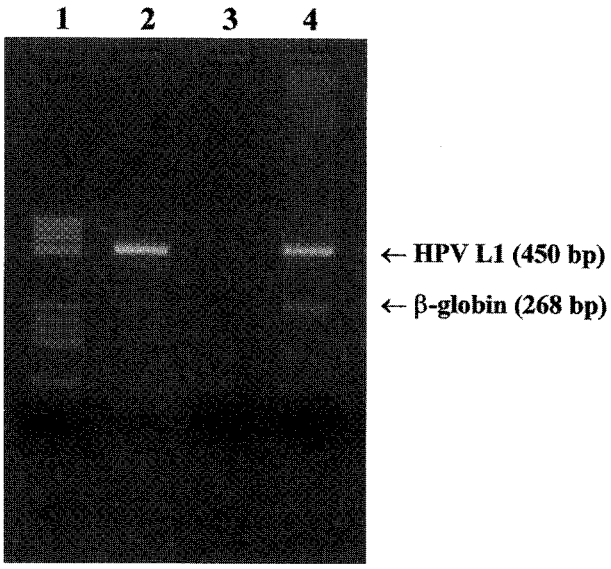

Fig. 1. — Agarose gel electrophoresis of products amplified using L1 consensus primers MYO9-MY1 1, visualised under W fluorescence after ethidium bromide staining. Lane 1 : molecular DNA weight marker. Lane 2 : positive control (CIN3 lesion proven to contain HPV16). Lane 3 : negative control (water). Lane 4 : pT3 NO ESCC, HPV positive, in a 64 years old male patient with no recorded history of warts or genital condylomas.

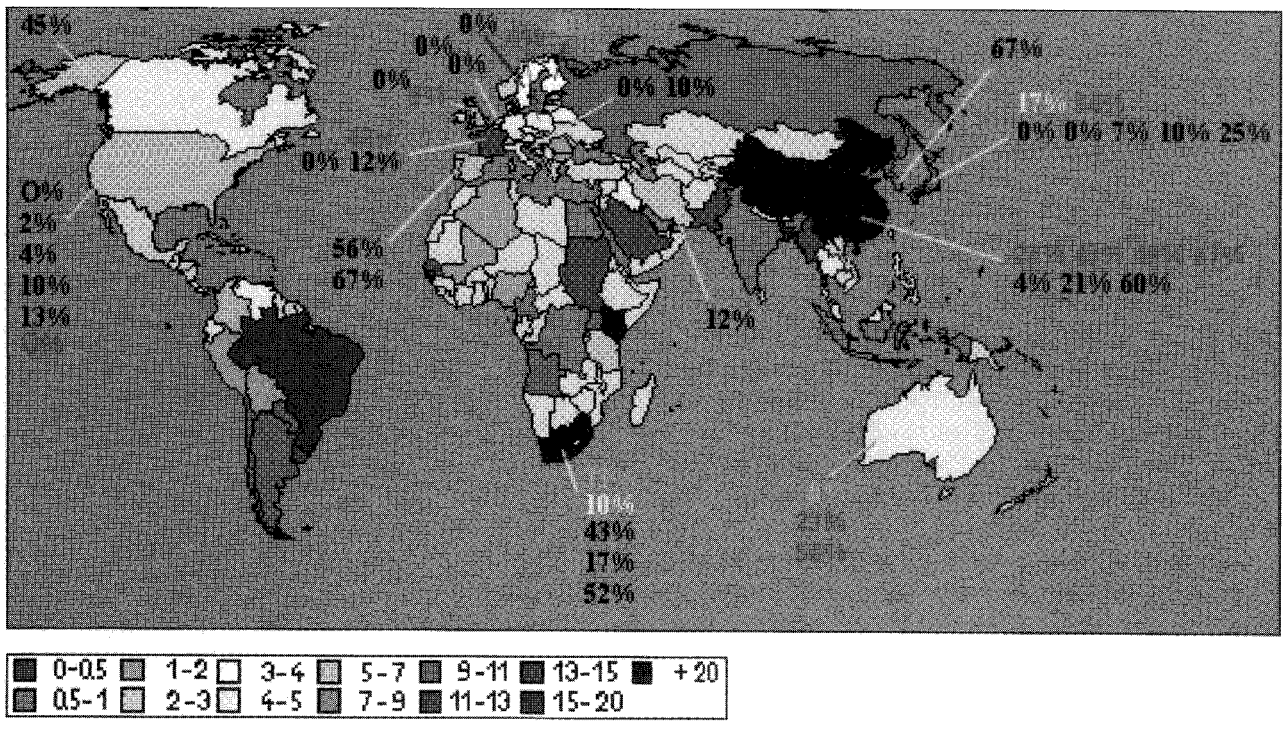

Fig. 2. — Map of oesophageal carcinoma world-wide incidence (per 100.000) (20) along with the percentage of HPV-positive ESCC found by the previous studies according to the technique used in each of them (4) (morphology/green, southern blot/red, immunohistochemistry/ yellow, in-situ hybridisation/magenta, PCR/black).

In the study by Fidalgo *et al.* (8), morphology using koïlocytosis as sole criteria failed to reveal the presence of HPV in half of the infected tumours when compared to PCR. No histological appearance close to these of cervical HPV lesions, was observed by Kluski *et al.* (9) in 120 OSCC cases while they found 5 HPV-positive cases in the 10 samples analysed by filter in-situ hybridisation. False positive were also observed. Morphology appears to lack both sensitivity and specificity m comparison to DNA detection technique and should not be used alone to asses HPV infection.

DNA detection techniques are much more sensitive than morphology. In-situ hybridisation can detect up to 1000 viral copies per celL while PCR (10-12) can detect up to 1000 viral copies present in the whole sample (for memory one koïlocyte contains 1000 to 10000 viral DNA). Both are prone to false-positive results due to their high sensitivity. Great precautions must be taken during PCR technique to avoid cross-sample contamination. False negative results can be traced in PCR using ß-globin gene amplification. PCR seems the best way to detect HPV infection when all precautions are taken to ensure minimal cross-contamination (7). High stringency conditions and internal ß-globin amplification should be used to avoid respectively false positive and false negative results.

Not all discrepancies in HPV prevalence can be explained by the relative performances of the various detection techniques used. Environmental factors probably play a role as shown by different results arising with the same technique in different geographical regions.

In regions with high incidence of OSCC, such as the so- called "Asian oesophageal cancer belt" and certain parts of Africa, showing a high prevalence of HPV infection, the virus could play an important role in the process of carcinogenesis (12,14-16). On the contrary, as shown by Poljak *et al.* (4) in a recent literature review, HPV presence is rare and its implication dubious in countries such as Europe and North America (11,17-20) where OSCC incidence is low.

The incidence of OSCC in Belgium is low (21), as in most regions of Europe and the 4,8% of HPV-positive OSCC we found are consistent with the results of other studies performed in similar countries. According to these findings, it seems HPV infection plays at best only a minor role — and maybe no role at all — in the pathogenesis of OSCC in Belgium as in other OSCC low risk area.

Moreover, as the majority of our patients were tobacco smokers, drinking more the 50g alcohol a day, or both, the main risk factors for OSCC development in our countries are still (1) to our knowledge alcohol abuse and smoking, especially when combined.

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