Original Article

Role of the Human Papillomavirus Infection in Esophageal Squamous Cell Carcinoma

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ABSTRACT

Background and Objective: **Human papillomavirus (HPV) is one of the possible etiologic factors in development of esophageal squamous cell carcinoma (ESCC). In this study we aimed to study the role of HPV in ESCC.**

Patients and Methods: **In this study, 140 cases of ESCC were analyzed for the HPV DNA by polymerase chain reaction (PCR) using GP5+/GP6+ primers for L1 open reading frame (ORF) to amplify a 150-bp segment of HPV L1 ORF. This region was subsequently sequenced to identify the type of HPV.**

Results: **A total of 140 patients enrolled in our study. In this respect, 50.7% of them were females and 49.3% were males, aged between 20 and 81 years old. In addition, 33 tumor specimens (23.6%) and 12 (8.6%) non-involved tumor margins were HPV positive. In HPV positive tumor cases, 36% were also positive in tumor margins. The HPV positive cases were 21.7% males and 25.3% females. There was no correlation between the presence and types of HPV with patients' sex and age. The frequency of HPV subtypes in tumoral regions were as follow: HPV-16: 60.6%, HPV-18: 30.3%, HPV-33: 6.1%, and HPV-31: 3 %. We found only HPV-16 in tumor margins.**

Conclusion: **Our results support a causal association between HPV infection and ESCC which is consistent with HPV studies conducted in other high-risk areas.**

Key words: Esophageal squamous cell carcinoma, Human papillomavirus, Polymerase chain reaction

Introduction

Esophageal cancer in humans occurs worldwide with a variable geographic distribution and ranks eighth in order of cancer occurrence, considering both sexes (1). Cigarette smoking and excessive alcohol intakes are two important and well known risk factors (2). In western countries, where the risk of ESCC is generally low, consumption of tobacco and alcohol

Received: 15 September 2006
Accepted: 22 December 2006
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could explain more than 90% of the cases of ESCC (3). However, in countries with the highest rates of ESCC, such as Iran and China, only a small proportion of ESCC cases could be attributed to smoking or alcohol consumption (4). Other factors that are thought to contribute to the pathogenesis include consumption of salt-pickled, salt-cured, and moldy foods, as these foods are frequently contaminated with N-nitrosamine carcinogens and/or fungal toxins (5). Consumption of hot beverages such as tea and fungal invasion in esophageal tissues leading to localized inflammation and irritation have been suggested as additional promoting factors for cancers of the esophagus (5).

However, these factors alone do not explain the rates of esophageal cancer seen in countries such as Iran and China. Microbial agents, especially HPV, may be one of the factors that explain part of this high incidence of ESCC. The role of HPV in ESCC has been studied in many high-risk and low-risk areas of the world (6-7). Most studies from high-risk areas such as China and South Africa have suggested a role of HPV in ESCC, while most studies from low-risk areas have failed to find any association (6-7). HPV types 6, 11, 16, 18, 31, and 33 represent the most common types found in the epithelium of squamous cell hyperplasia, dysplasia, and carcinomas (8-9). HPVs can be categorized as either high-risk types (HPV-16, 18, 31, and 33) or low-risk types (HPV-6, 11) (9-10). Members of the high-risk group promote carcinogenesis and their DNA usually integrates into the host genome, whereas, the low-risk HPV types are primarily found in benign tumors and their DNA remain extra chromosomal. HPV-6, 11, 16, 18, 31, and 33 have been described in association with esophageal squamous cell lesions (10-11). However, the positive incidence of HPV varies significantly depending on the geographical location of the patient (12-13). In this cross sectional study, we aimed to evaluate whether the infection with HPV may be a factor in the development of ESCC and determined the frequency of HPV and its types (16, 18, 31, and 33) in ESCC in Iranian patients.

Patients and Methods

In this study, a total of 140 patients with dysphagia who admitted in gastroenterology wards with clinical signs suspicious for esophageal carcinoma were underwent upper GI endoscopy. Three biopsies, one from the suspicious lesion, one from non-involved upper margin and one from non-involved lower margin were taken. These biopsies were fixed in 10% buffered formalin. Formalin fixed specimens were processed routinely, and embedded in paraffin. For each case, all available hematoxylin and eosin stained sections were reviewed for confirmation of squamous cell carcinoma and a representative block was selected for further studies.

Polymerase chain reaction assay

After confirmation of squamous cell carcinoma by a pathologist, 5-10 μm thick sections were prepared from each specimen for DNA extraction. Measures were taken to avoid any cross-contamination between samples (using separate disposable items such as gloves, blades, and tubes; most importantly the first section of each specimen plus gloves and blade were discarded and new blade and gloves were used for main sectioning). Sections were subsequently deparaffinized by xylene and digested using digestion buffer containing proteinase K, followed by extensive extraction with phenol/chloroform. The extracted DNA was stored at 4°C until tested. Extracted DNA from HeLa cell line was used as HPV-positive control. No DNA was added for negative controls.

DNA quality was evaluated by PCR using primers PCO3/PCO4 that amplify a 110 bp product from the human β-globin gene. β-globin positive samples were subjected to HPV PCR by GP5+/GP6+ primers for L1 open reading frame (ORF) that amplifies a 150 bp product from the HPV L1 ORF.

PCR was performed according to the procedure described by Yi Ting et al. Samples were subsequently subjected to agarose gel electrophoresis (2% agarose) and stained with ethidium bromide. Subsequently, sequencing of PCR products were done by Macrogen (Seoul, South Korea) facility to reveal the type of HPV. GenBank accession numbers for our nucleotide sequences are as follows:

IR-ESO-HPV-16/1 DQ448182
IR-ESO-HPV-16/2 DQ448183
IR-ESO-HPV-16/3 DQ448184
IR-ESO-HPV-16/4 DQ448185
IR-ESO-HPV-16/5 DQ448186
IR-ESO-HPV-16/6 DQ448187
IR-ESO-HPV-16/7 DQ448188
IR-ESO-HPV-16/8 DQ448189
IR-ESO-HPV-16/9 DQ448190
IR-ESO-HPV-16/10 DQ448191
IR-ESO-HPV-16/11 DQ448192
IR-ESO-HPV-16/12 DQ448193

IR-ESO-HPV-16/13 DQ448194
IR-ESO-HPV-16/14 DQ448195
IR-ESO-HPV-16/15 DQ448196
IR-ESO-HPV-16/16 DQ448197
IR-ESO-HPV-16/17 DQ448198
IR-ESO-HPV-16/18 DQ448199
IR-ESO-HPV-16/19 DQ448200
IR-ESO-HPV-16/20 DQ448201
IR-ESO-HPV-18/1 DQ448202
IR-ESO-HPV-18/2 DQ448203
IR-ESO-HPV-18/3 DQ448204
IR-ESO-HPV-18/4 DQ448205
IR-ESO-HPV-18/5 DQ448206
IR-ESO-HPV-18/6 DQ448207
IR-ESO-HPV-18/7 DQ448208
IR-ESO-HPV-18/8 DQ448209
IR-ESO-HPV-18/9 DQ448210
IR-ESO-HPV-18/10 DQ448211
IR-ESO-HPV-31 DQ448212
IR-ESO-HPV-33/1 DQ448213
IR-ESO-HPV-33/2 DQ448214

Statistical analysis

Our data were collected and analyzed using Epi Info software (version 3.3.2). Chi-square test was used for the analysis of presence of HPV in tumoral region and non-involved tumor margins and the type of HPV.

A P value of less than 0.05 was considered to indicate statistical significance.

Results

Out of 140 confirmed ESCC enrolled in our study, 50.7% of the patients were females and 49.3% were males, aged between 20 and 81 years old. 50% of all patients were between 60-70 years. Thirty-three tumor specimens (23.6%) and 12 (8.6%) non-involved tumor margins were HPV positive ($p<0.00001$). From HPV positive tumor cases, 36% were also positive in non-involved tumor margins. The distribution of the HPV positively in cases with esophageal cancer, based on sex of the patients, was 21.7% for males and 25.3 % for females. There was no significant correlation between presence and types of HPV with patients' sex and age. The frequency of HPV subtypes in tumoral regions were as follow: HPV-16: 60.6%, HPV-18: 30.3%, HPV-33: 6.1%, and HPV-31: 3%; and this difference was significant ($p<0.00000003$). We found only HPV-16 in tumor margins of HPV positive cases.

Discussion

Esophageal cancer is eighth frequent neoplasm among neoplasms. It is more frequently seen in males. In high incidence areas, the male to female ratio is low. Esophageal carcinoma has a distinct geographical distribution with a high prevalence in certain regions of Iran, China, Africa, and France (14-17). The basis for the variation in geographical distribution of the disease stems in part from environmental factors such as the mineral content of the soil, dietary practices, occupational factors, and personal habits (17). The current data suggest that the multifactorial etiology of esophageal cancer differs between the low- and high-incidence geographic areas (18). In western countries, where the risk of ESCC is generally low, consumption of tobacco and alcohol could explain more than 90% of the cases of ESCC (3). However, in countries with the highest rates of ESCC, such as Iran and China other risk factors such as microbial agents, especially HPV, may be one of the factors that explain part of this high incidence of ESCC (5). Meanwhile, HPVs are oncogenic viruses and show oncogenic activity through spoiling mucosal immune resistance and destroying tumor suppresser genes (15). HPV was found to be associated with both benign and malignant lesions developed in many sites of the body including the skin, aero-digestive tract, oral cavity, colorectal, and anal region (19). There is increasing evidence suggesting that HPV infection (primarily with HPV type 16 and 18) is an important etiologic factor in esophageal cancers (20-21). An association of HPV with esophageal carcinoma has been previously reported in many countries. The incidence of infection differs markedly depending on the different geographic location of the population under study and within different studies (21-22). HPV prevalence bears a close correlate to the incidence of SCC, being low (0-3%) and high (up to 80%) in the respective geographic regions (18). In China the frequency of HPV in ESCC were reported between 6.7% and 83.3% in different parts of this country (20). There was high incidence of HPV infection in the esophageal epithelium in Eastern Guangdong, Southern China, where esophageal carcinoma is prevalent. HPV has been seen in the normal, Paracancerous, and cancerous tissues, with the high-risk HPV type 16 and 18 more common in cancerous tissues. The results indicate that the high incidence of esophageal carcinoma in this area is associated with HPV infection (21). In one study in South Africa 71% of patients with esophageal carcinoma were positive for HPV DNA either in the tumor biopsies

or in adjacent tissue(22) and in another study HPV were detected in 52% of esophageal cancers and HPV 16 was present in 84% of the HPV-positive cancers(23). In Japan most oncogenic types of HPV (HPV 16 and HPV 18) were detected by PCR in carcinomas of the esophagus (24). In a recent study in Iran 36.8% of the ESCC samples were positive for HPV. In this regard, 13.2% and 7.9% of these samples were positive for the HPV-16 and HPV-18 respectively (25). In another study the HPVs frequency in ESCC were as follow: HPV-16 (54.7%), HPV-18 (4.8%), HPV-6 (14.3%), HPV-66 (7.1%), HPV-52 (4.8%), and 14.3% of cases were positive for more than one type of HPV (26). In western countries the results show that in contrast to geographic regions where ESCC is prevalent, HPV infection occurs infrequently in association with ESCC (27). Our results are consistent with HPV DNA studies conducted in other high-risk areas for ESCC which showed evidence of HPV in tumor tissues from 20% to 50% of ESCC cases and provided further evidence to support a causal association of HPV infection with esophageal squamous cell carcinoma. Also in our study, HPV-16 was the most prevalent type of HPV among the esophageal cancer cases together with HPV-18, as reported in most other high-risk areas.

Conclusion

Our results support a causal association between HPV infection and ESCC and are consistent with HPV studies conducted in other high-risk areas.

Acknowledgment

The authors are grateful to Pasteur Institute of Iran for financial support of this study. The authors wish to thank Miss Jaleh Taeb for her contribution in writing this work.

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