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Original article

Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an instutional experience from Turkey and review of literature

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ABSTRACT

Esophageal cancer is a poor-prognosis malignancy that ranks eighth among all cancer types, and its prevalence shows differences among geographical regions. Although the most important risk factors for esophageal carcinoma are alcohol and smoking, viral infections, particularly HPV infection, are also considered among etiological agents. Our study aims to detect the presence of HPV in esophageal cancers in our patient population and to investigate its correlation with clinico-pathological parameters.

We investigated the presence of HPV-DNA by real-time polymerase chain reaction in a total of 52 patients with esophageal cancer. Subtype analysis was performed in positive cases and was correlated with selected clinico-pathological parameters.

Five (9.6%) of 52 tumor samples, 3 squamous cell carcinomas (3/33 cases) and 2 adenocarcinomas (2/19 cases), were HPV-DNA-positive. Subtype analysis could be performed in four HPV-DNA-positive cases, of which three were HPV type-39 and 1 was type-16. The Marmara region, where the present study was carried out, is a region with low-moderate risk for esophageal cancer, and the prevalence of HPV-DNA in these tumors is similar to the prevalence of HPV-DNA reported in the literature for regions with similar risk.

In conclusion, we detected HPV DNA in a subset of esophageal and gastroesophageal junction tumors. HPV infection may have a role in esophageal carcinogenesis and high-risk HPV subtypes can particularly be considered among risk factors since the prevalence of high risk HPV infection has also been found to be increased in regions with a high risk for esophageal cancer compared to low-moderate risk regions.

Introduction

Esophageal cancer is a rapidly progressive and poor-prognosis disease with late manifestation [1]. It ranks eighth among all types of cancer with a prevalence rate of 4.2% [2,4]. The most common tumor type of esophageal cancer is squamous cell carcinoma (SCC) followed by adenocarcinoma [2,5,6]. These two tumor types account for the great majority of esophageal cancers, and there are significant epidemiological/etiological differences between them [2,3,7]. Whilst SCCs are more prevalent in the eastern countries and developing countries, the incidence of adenocarcinomas, which were less prevalent in the recent years, increases by 5–10% each year particularly in the industrialized western countries [3]. This tumor, which appears at the age of 65 years in average, shows male predominance particularly for adenocarcinomas. Its incidence shows variation among countries, as well as among geographical regions in the same country. Countries where the disease is more prevalent include China, Japan, Korea, India, Singapore, South Africa, Russia, Turkmenistan and Iran. Esophageal cancer, which has low-moderate risk in Turkey, is more prevalent especially in the Eastern Anatolian Region [3,7,8]. The etiology of

Abbreviations: SCC, squamous cell carcinoma; GER, gastro-esophageal reflux; BE, barrett's esophagus; HPV, human papillomavirus; RT-PCR, real-time polymerase chain reaction.

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esophageal carcinomas includes tobacco and alcohol consumption, local environmental and nutritional carcinogens, vitamin and mineral deficiencies, achalasia, tylosis, caustic stenosis, celiac disease, Plummer–Vinson syndrome, gastro-esophageal reflux (GER), Barrett's esophagus (BE) and Human Papillomavirus (HPV) [5,8].

HPV is worldwide one of the most common sexually-transmitted agents in both females and males. The exact incidence and prevalence are not known since it is not a disease of mandatory reporting. It is agreed that HPV infection is an effective factor in the carcinogenesis of cervix uteri, skin, oral cavity, pharynx, larynx, and anogenital system tumor [6,9,10]. Although the relation between HPV infection and esophageal cancer has been identified in many regions of the world, the role of HPV infection in the etiology of esophageal cancer is not clear in Turkey, particularly in our region.

The present study aimed to detect the prevalence of HPV-DNA in the esophageal cancers in our region, to determine which types are more prevalent in the patients from whom HPV-DNA was isolated, and to evaluate the relation with clinico-pathological data.

Material and method

Patients

Hematoxylin & Eosin stained preparations of a total of 52 cases, of which esophagus and gastric resection materials had been examined between 2000 and 2013 at Kocaeli University Faculty of Medicine, Department of Pathology and 33 had been diagnosed with SCC whereas 19 had been diagnosed with adenocarcinoma, were retrospectively evaluated. Averagely 10 sections in 5-micron thickness were obtained from the selected paraffin blocks and were examined to detect the presence of HPV infection by real-time polymerase chain reaction (RT-PCR) technique, and then type of the virus in these cases was assessed. In addition, eight esophageal mucosa samples with nonspecific changes of patients who were operated due to non-tumoral diseases were included in the study as the control group. The relation between presence of HPV infection and certain prognostic factors was evaluated. Clinical information about patients (age, gender) was derived from the patient files, whereas prognostic parameters (tumor localization, macroscopic size of the lesion, histologic grade of tumor, lymph node metastasis, and depth of invasion) were retrospectively derived from the pathology reports.

HPV-DNA isolation

HPV-DNA was isolated using magnetic particle technology (QIAsymphony SP, Qiagen GmbH, Hilden, Germany).

HPV RT-PCR

HPV PCR was performed on real-time platform using Bosphore HPV Detection Kit v1 (Anatolia Geneworks, Istanbul, Turkey).

HPV genotyping

Purification procedure was performed in the amplified samples using High Pure PCR Product Purification Kit after PCR. Thereafter, they were involved in sequencing reaction in the presence of sequencing primary (GP5 or GP6) and Sequence Reagent Mix DYEnamic ET Terminator Cycle Sequencing Kit (Roche Diagnostics GmbH, Mannheim, Germany). For this purpose, 0.6 μl sequencing primary was used. Sequencing reaction was performed under the conditions of 95 °C/20 s, 50 °C/25 s and 60 °C/2 min at 35 cycles. Samples for DNA sequencing were loaded into ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc., California, USA). Electropherogram of the sequence was obtained by Vector NTI v5.1 (InforMax, Invitrogen life science software, Frederick, MD 21704, USA) and was defined by sequence BLAST analysis (Basic Local Alignment Search Tool, BLASTN program 2.2.25, blast.ncbi.nlm.nih.gov).

Statistical analysis

"SPSS (Statistical Package for the Social Sciences) for windows 20.0" package program was used for the statistical analysis of data. Results were presented as mean ± std. deviation. Whether there is difference between the groups was assessed by Chi-Square test. The difference was considered significant in case of a $p < 0.05$.

Results

Of the 60 cases enrolled in the study, 33 had been diagnosed with SCC and 19 had been diagnosed with adenocarcinoma, whereas remaining eight were the control cases. Of the overall cases with esophageal and gastroesophageal region carcinoma, 19 (36.5%) were female, 33 (63.5%) were male, and the mean age was 61.92 ± 11.72 years (range: 20–78 years); 59.6 ± 13.1 years for the cases with SCCs and 65.95 ± 7.5 years for the cases with adenocarcinomas. The mean tumor size was 4.96 ± 2.38 cm.

With regard to the distribution of overall lesions among localizations, it was observed that the most common localization of SCCs was the lower part of esophagus with 21 cases (63.6%) followed by middle part with 11 cases (33.3%) and upper part with 1 case (3.1%). All of the cases diagnosed with adenocarcinoma were localized in the inferior end of esophagus and proximal stomach (gastric cardia). According to Siewert classification, one case (5.2%) was Type I (inferior end), five cases (26.3%) were Type II (GEJ) and 13 cases (68.5%) were Type III (gastric cardia).

HPV-DNA positivity was detected in five (9.6%) of a total of 52 cases with esophageal cancer; in three (9.1%) of 33 SCC cases and in two (10.5%) of 19 adenocarcinoma cases. HPV-DNA was not isolated from any of the eight control cases (Table 1). Statistical analyses revealed no significant difference between SCC and adenocarcinoma groups and the control group in terms of the rate of HPV-DNA isolation ($p > 0.05$).

RT-PCR performed with primaries that were specific to HPV types revealed five tumor tissue samples that were positive for HPV-DNA (Fig. 1) (Table 2). Four of HPV-DNA-positive tissue samples could be typed. Accordingly, HPV type-39 was isolated in two (H21 and H22) of three HPV-DNA-positive cases diagnosed with SCC, whereas typing could not be performed in one SCC case (H54). Whilst, HPV type-39 was isolated in one (H68) of two HPV-DNA-positive cases diagnosed with adenocarcinoma, HPV type-16 was

Table 1
Presence of HPV-DNA in the esophageal SCC and adenocarcinoma and the control group.

Diagnosis	HPV		Total
	(−)	(+)	
SCC	30 (90.9%)	3 (9.1%)	33
Adenocarcinoma	17 (89.5%)	2 (10.5%)	19
Control group	8 (100%)	0 (0%)	8
Total	55	5	60

Table 2
HPV positive cases and HPV types.

Case no	Tumor type	HPV type
H21	Squamous cell carcinoma	Type 39
H22	Squamous cell carcinoma	Type 39
H54	Squamous cell carcinoma	Not available
H68	Adenocarcioma	Type 39
H41	Adenocarcioma	Type 16