



## Analyzing esophageal squamous cell papillomas for the presence of human papilloma virus

Arzu Tiftikçi<sup>1</sup>, Eser Kutsal<sup>1</sup>, Ender Altıok<sup>2</sup>, Ümit İnce<sup>3</sup>, Bahattin Çicek<sup>1</sup>, Murat Saruç<sup>1</sup>, Nurten Türkel<sup>1</sup>, Özdal Ersoy<sup>1</sup>, Güven Yenmiş<sup>2</sup>, Nurdan Tözün<sup>1</sup>

<sup>1</sup>Department of Gastroenterology, Acibadem University School of Medicine, İstanbul, Turkey

<sup>2</sup>Acibadem Genetic Diagnosis Center, İstanbul, Turkey

<sup>3</sup>Department of Pathology, Acibadem University School of Medicine, İstanbul, Turkey

### ABSTRACT

**Background/Aims:** Human Papilloma Virus (HPV) infection can be a predisposing condition for the development of squamous cell papilloma (SCP) of the esophagus, which can progress to dysplasia and to carcinoma as a result of chronic infection. The aim of the present study was to search for the presence of HPV in the esophageal SCP, and to genotype the detected HPV.

**Materials and Methods:** Data from patients with definite diagnosis of SCP of the esophagus were identified from pathology records for two years period at different Hospitals. Slides from each patient were reviewed and samples with satisfactory papilloma tissues were submitted to molecular analysis. DNA has been isolated. DNA sequencing has been performed for genotyping HPV for all types.

**Results:** Our study group consisted of 21 women and 17 men (a total of 38 patients), mean age was 41 years (range 17-67 years). Most of the papillomas were located at mid-esophagus (68%). Eight out of 38 patients (21%) had associated erosive esophagitis, and fourteen patients (36.8%) had *Helicobacter Pylori* (*H. pylori*). Of the 38 SCP analyzed, seven (19%) were positive for HPV DNA. Three of them were of genotype 6, whereas four were of genotype 16,18,31,81 that are known as highly oncogenic. There were no correlations between the presence of HPV and the patient's age, the presence of reflux esophagitis or *H. pylori*, smoking habit and the location of the papillomas.

**Conclusion:** The presence of high-risk type HPV in esophageal SCP may implicate a role of the virus in the pathogenesis of the esophageal tumor.

**Keywords:** Esophagus, genotype, human papilloma virus

### INTRODUCTION

Globally, esophageal cancer is the eighth most common cancer and the sixth most common cause of death due to cancer (1). Although the incidence of adenocarcinoma is increasing in developed countries, the type of esophageal carcinoma worldwide is mainly squamous cell carcinoma, with a marked geographic and ethnic variation in the incidence (2). Consumption of food and water rich in nitrates and nitrosamines, mycotoxins, alcohol and tobacco usage, radiation exposure, deficiency of vitamins A, B, C, and trace elements, achalasia, chronic strictures resulting from acid or lye ingestion, and genetic factors have all been reported as risk factors for the development of esophageal squamous cell carcinoma

(3). In addition, human papilloma virus (HPV) infection is a predisposing factor (4). HPV could be a causative agent for the occurrence of squamous cell papilloma (SCP), which is followed by dysplasia and then carcinoma that can be the result of chronic infection. In light of this knowledge, it is not known how much SCP occurrence could be related to the presence of HPV. In many studies, the presence of HPV in squamous papilloma has been shown (4,5). In a Canadian study, a total of 21 squamous papilloma samples were studied and 12 were HPV positive (57%), with majority being genotype 16 (5). The aim of the present study was to investigate the presence of HPV and determine its genotype in the prediagnosed SCP of the esophagus.

This study was presented in European Gastroenterology Week in 2011 as a poster.

**Address for Correspondence:** Arzu Tiftikçi E-mail: arzutiftikci@hotmail.com

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**MATERIALS AND METHODS**

Cases from a 2-year period with a pathological diagnosis of esophageal SCP were obtained from Pathology Department files at different hospitals of our group. Histology slides from each case were reviewed, and the cases with a satisfactory papilloma tissue were selected for molecular analysis. Subsequently, DNA was isolated from sections of formaldehyde-fixed paraffin-embedded tissues, obtained during esophagogastroduodenoscopy procedures seen as small whitish sessile growth in the esophagus (Figure 1). Histological examination of tissues had revealed characteristic finger-like projections of tissue lined by an increased number of squamous cells and by the uninflamed fibrovascular core containing small blood vessels with conserved cellular orientation and normal differentiation without signs of cytological atypia (Figure 2). The locations of the papillomas were designated as the upper esophagus (<24 cm from the incisors), middle (24-32 cm from the incisors), or lower (>32 cm from the incisors). Demographic and clinical data of each patient were retrieved from corresponding clinical records. The procedures followed were in accordance with the Helsinki Declaration. Polymerase chain reaction and capillary DNA sequencing using MY09-MY11 and GP5-GP6 primer pairs were performed for genotyping of all HPV types. Homologies of DNA sequences were compared against HPV databases. Beta-globin primers were used as internal controls for DNA isolation and amplification. Only beta-globin-positive samples in the absence of HPV DNA amplification were concluded as HPV negative.

**Statistical Analysis**

The Statistical Package for Social Sciences (IBM SPSS Statistics; Armonk, NY, USA) version 21 software was used to analyze the data. Fisher exact and chi-square tests were used as statistical methods.

**RESULTS**

Our study group consisted of 21 women and 17 men (n=38 patients), with a mean age of 41 years (range, 17-67 years). Twenty-one patients were smokers (55%). The location of papillomas was mid esophagus in 26 (68%) patients, distal in 8 (21%), upper in 4. The sizes of the polyps were an average of 3 mm (range, 2-8 mm). Eight out of 38 polyps (21%) had associated erosive esophagitis, and 14 (36.8%) additionally had *Helicobacter pylori* infection. Of the 38 SCP cases analyzed, 7 (19%) were positive for HPV DNA (5 males and 2 females). Three cases were genotype 6, whereas 4 were the highly oncogenic genotypes of 16, 18, 31, and 81. One of the high oncogenic risk papillomas contained 2 high-risk genotypes concurrently. Among the 4 high-risk HPV patients, 3 were males. Out of 7 HPV-harboring patients, 2 were smokers: one at high and the other at low oncogenic risk. There was no correlation between the presence of HPV and the age of the patients, presence of reflux esophagitis, presence of *H. pylori*, smoking status, and location of papilloma (Table 1).

**DISCUSSION**

Esophageal squamous papilloma is a rare, asymptomatic, benign tumor of the esophagus. When detected at endoscopy,

complete removal is recommended in order to disregard the possibility of malignant transformation (6). There is a marked geographic and ethnic variation in the incidence. Most of the literature is from studies based in Europe (7,8). Local irritation caused by gastroesophageal reflux and HPV infections are the frequently reported causative agents.



Figure 1. Endoscopic appearance of squamous papilloma

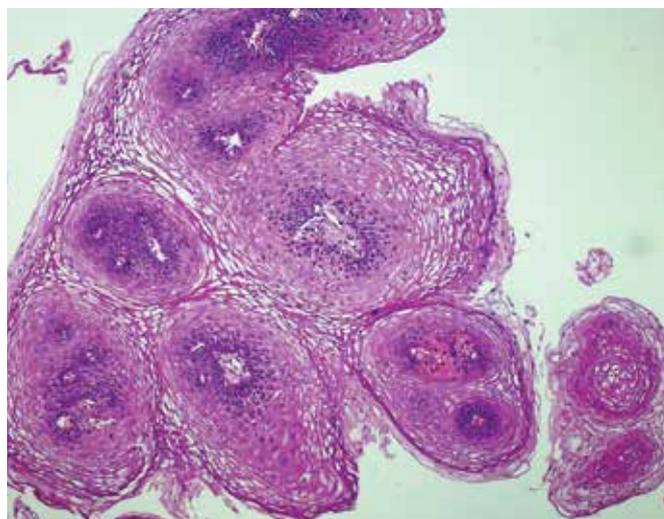


Figure 2. Squamous papilloma hematoxylin and eosin staining 100x

Table 1. Patient characteristics according to the HPV status

	HPV positive (n=7)	HPV negative (n=31)	p
Age, years, median (min-max)	47 (20-54)	38 (17-67)	>0.05
Female/male	5/2	19/12	
Smokers	4	17	>0.05
Presence of <i>H. pylori</i>	2	12	>0.05
Presence of esophagitis	1	7	>0.05
Location of squamous papilloma	Upper: 1 Middle: 5 Lower: 1	Upper: 3 Middle: 21 Lower: 7	>0.05

Generally, male patients are affected (5,6); however, in our study, female patients having SCPs were slightly predominant (55%); interestingly, predominantly male patients had HPV-positive papillomas (71%). In some studies, the presence of HPV in squamous papilloma has not been investigated (8,9), whereas in others, up to 50% of cases have reported HPV positive papillomas (5). In our study, 7 (19%) of the patient's SCPs were positive for HPV DNA.

Squamous cell papillomas of the esophagus are usually observed as single, whitish, small, elevated lesions and rarely as massive papillomatosis (10,11). Only one papillomatosis case was noted in our series and the remaining 37 cases presented as small, solitary, elevated lesions from the mucosa. Similar to other reports most of these papillomas (68%) were located at the mid esophagus. The average sizes of the polyps were 3 mm.

Severe erosive gastroesophageal reflux has been considered as an etiological factor of esophageal SCP, particularly if it was located distally (5,12). In our study, 8 out of 38 SCP cases (21%) had associated erosive esophagitis, and half of these were also HPV positive.

The oncogenic potential of each type of HPV is well described. It has been widely studied in gynecological cancers, mostly in cervical cancers. In esophageal SCP, genotype 6 HPV, which has lower oncogenic risk profile, is mainly detected (12-14). In our study, amongst the 38 SCPs analyzed, 7 (19%) were positive for HPV DNA (5 males and 2 females); 3 SCPs were of genotype 6, whereas 4 were of genotypes 16, 18, 31, and 81 that are known to have high oncogenic potential. Esophageal SCP with high oncogenic risk has been reported previously (15,16). In our study, one of the high oncogenic risk-containing papillomas presented 2 types of high-risk genotype within the same papilloma. Amongst the 4 high-risk HPV patients, 3 were males. Two out of 7 HPV-positive patients were smokers: one with high and the other with low oncogenic risk HPV.

There was no correlation between the presence of HPV and the age of the patients, presence of reflux esophagitis, presence of *H. pylori*, smoking habits, and location of papillomas.

The presence of high-risk HPV types in esophageal SCPs may implicate a role of the virus in the pathogenesis of the tumor; however, further studies with long-term follow-up are needed in order to confirm such an observation.

**Ethics Committee Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

**Informed Consent:** N/A.

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